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A new partition agent for use in the rapid separation of fatty acid esters by gas-liquid chromatography*

The standard methods for the separation of long-chain fatty acids by reversed-phase partition chromatography or paper chromatography have been supplemented or replaced by the procedure of gas–liquid chromatography recently developed by JAMES AND MARTIN[1]. By utilizing a paraffinic hydrocarbon (Apiezon "M") as a partition liquid, these investigators were able to separate the methyl esters of saturated and certain unsaturated acids of chain lengths up to 20 C atoms in 3–4 h. The separation of the C_{18} unsaturated acids was difficult. Frequently, the separation of linoleate from oleate was incomplete and, further, it was not possible to distinguish linolenate from linoleate under their experimental conditions.

With the intention of obtaining quicker, sharper, and more complete resolution, particularly of the aforementioned polyunsaturated acids, a series of different types of partition agents was investigated in this laboratory. The adipate polyester of diethylene glycol** proved to be an extremely efficient phase for the analysis of fatty acids. Due to the more polar nature of this material in comparison to the hydrocarbons previously used[1], two significant changes were noted. First, there was the complete elution of a standard mixture of methyl esters of fatty acids up to C_{22} from a column operating at a temperature of 186° within 85 min (Fig. 1). Second, a sufficient increase in retention times occurred (Fig. 2) as the degree of unsaturation increased, giving for the first time the complete resolution of each member of the C_{18} series from its preceding, less funsaturated member. ORR AND CALLEN[2] working with another relatively polar phase also noted avorable separations of this class of compounds.

The present investigation was carried out with a thermal conductivity device employing a split stream of helium through the reference and detection sides of the sensing element which

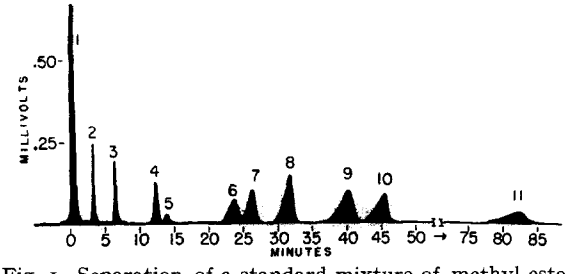

Fig. 1. Separation of a standard mixture of methyl esters from fatty acids. Load 2.5 mg. 1, air; 2, methyl laurate; 3, methyl myristate; 4, methyl palmitate; 5, methyl palmitoleate; 6, methyl stearate; 7, methyl oleate; 8, methyl linoleate; 9, methyl linolenate; 10, methyl arachidate; 11, methyl behenate.

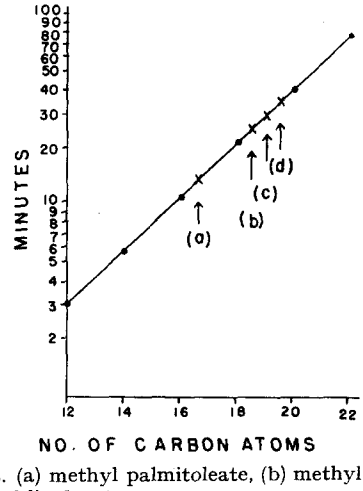

Fig. 2. Semi-logarithmic plot of retention time against number of C atoms for a standard mixture of methyl esters of fatty acids. ● – saturated esters, × – unsaturated esters. (a) methyl palmitoleate, (b) methyl oleate, (c) methyl linoleate, (d) methyl linolenate.

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** No. LAC-1-R296, obtainable in the 1 lb. containers from Cambridge Industries Company, Inc., 101 Potter Street, Cambridge 42, Mass., U.S.A.

was vented to the atmosphere. An 8 ft. glass "U" shaped column with an internal diameter of 6 mm containing one part of the alkyd resin to four parts of supporting material (acid-washed Celite 545, 120–140 mesh) was maintained at 186°. The flow rate of helium was 40 ml/min at 38 lb/in.^{2} pressure.

By utilizing various members of the alkyd resins as partition agents, it now appears possible to obtain good resolution of the individual components of mixtures of fatty acid esters of chain length up to at least C_{28} within a reasonable period of time. This would involve altering the parameters of column length, gas flow, temperature and mesh size. A more detailed report concerning these experiments will be forthcoming shortly.

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Some effects of thyroxin on oxidative phosphorylation in submitochondrial particles and intact mitochondria

Although thyroxin has been found by many[1,2,3] to cause uncoupling of oxidative phosphorylation in liver mitochondria, these effects have usually required some form of damage to the mitochondrial structure, and TAPLEY, COOPER AND LEHNINGER[3] were unable to observe uncoupling using submitochondrial particles obtained by digitonin treatment of mitochondria. It is the purpose of this communication to report that thyroxin will cause an increase in the P:O ratios obtained with either intact mitochondria or submitochondrial particles and that under conditions where the rate of oxidation is not limited by the rate of phosphorylation this increase in P:O ratio occurs together with a marked increase in the rate of O_2 uptake. Secondly, various forms of pretreatment of either intact mitochondria or submitochondrial particles lead to marked inhibition of phosphorylation by thyroxin, and under these circumstances there is no increase in rate of oxidation.

Mitochondria were prepared from rat liver[4,5] and submitochondrial particles were prepared by sonic treatment[6]. O_2 uptake was measured with the Clark O_2 electrode and P uptake with the ^{32}P-incorporation procedure[6,7].

Table I shows that, using intact mitochondria with succinate as a substrate, the P:O ratio was increased by thyroxin in experiments started by adding the mitochondria to a complete reaction medium. The increase in P:O ratio was accompanied by a decline in the rate of O_2 uptake. When the mitochondria were preincubated in the reaction medium for 4 min prior to addition of thyroxin and substrate, a marked inhibition of phosphorylation was evident, but this "uncoupling" was accompanied by a drop in the rate of O_2 uptake.

The second part of the Table shows results obtained with thyroxin using submitochondrial particles. For both substrates, thyroxin caused an increase in P:O ratio, and this was accompanied by a rise in the rate of oxidation. Preincubation of the particles in the reaction medium for 4 min followed by the addition of thyroxin and then either succinate or DPNH* resulted in a marked inhibition of phosphorylation compared with the preincubated control.

In addition to thyroxin, triiodothyronine**, diiodothyronine** and tetrachlorothyronine** were tested with both mitochondria and submitochondrial particles. Of the three thyroxin analogues only triiodothyronine was found to have appreciable activity and it was less effective than thyroxin. The effects observed therefore appear to be specific for thyroxin.

The experiments with thyroxin described above seem to be best explained in terms of three effects. One is the increase in the efficiency of the phosphorylation process that was evident with both intact mitochondria and submitochondrial fragments. In other experiments it was found that this increase in efficiency was not due to an inhibition of ATPase activity. A second effect of thyroxin was to increase the overall rate of oxidation. This did not occur with intact mitochondria but was evident with a variety of substrates using the more loosely coupled submitochondrial particles and has also been apparent in much early work with whole tissues and intact animals[8].

* The following abbreviations are used: ATP for adenosine triphosphate; ADP for adenosine diphosphate; AMP for adenosine monophosphate; DPNH for reduced diphosphopyridine nucleotide; P for inorganic orthophosphate.

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