Eiern nachweisen. An befruchteten Eiern ist das Ausmass der Abnahme des O_2-Verbrauchs grösser. dTC besitzt demnach neben seiner bekannten elektiven Wirkung auf die Endplatten der motorischen Nerven[2] auch

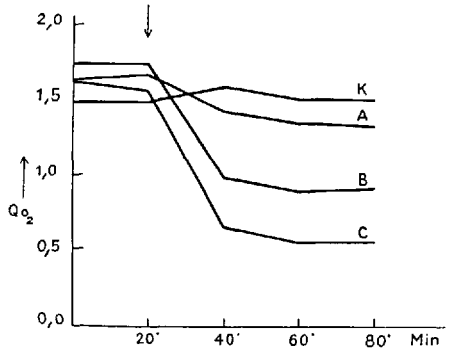

Abb. 1. O_2-Verbrauch der unbefruchteten Eier von *Psammechinus microtuberculatus* nach der Einwirkung von dTC. *K* Kontrolle; *A* 1,2 mg% dTC; *B* 5 mg% dTC; *C* 7,5 mg% dTC. Versuch bei 21°; Einkippen der Pharmakalösung bei ↓.

eine direkte Wirkung auf die Einzelzelle. Prostigmin bzw. Mestinon, welche die Curarewirkung an der motorischen Endplatte bekanntlich aufheben[3], vermögen die durch dTC hervorgerufene Senkung des O_2-Verbrauchs nicht zu antagonisieren.

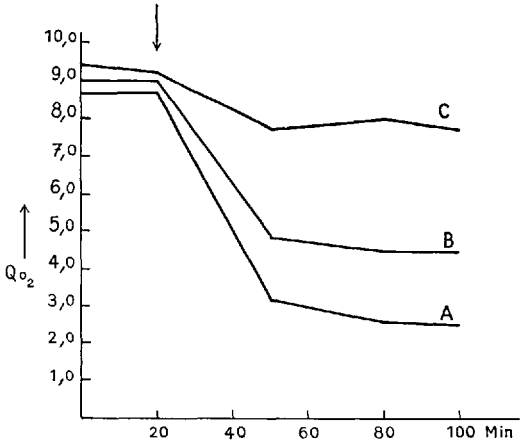

Abb. 2. O_2-Verbrauch der befruchteten Eier von *Psammechinus microtuberculatus* nach dTC. *A* 7,5 mg%; *B* 5 mg%; *C* 1 mg%. Versuch bei 21°.

Vitalfärbeversuche mit Neutralrot deuten darauf hin, dass die durch dTC hervorgerufene Senkung des O_2-Verbrauches durch eine Verminderung der Grenzflächendurchlässigkeit hervorgerufen wird. Ausführliche Darstellung und Diskussion der Ergebnisse an anderer Stelle[4].

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δ-Tubocurarinchlorid wurde uns freundlicherweise von der Firma Chemische Fabrik Asta (Brackwede), Prostigmin und Mestinon von der Firma Hoffmann La-Roche (Grenzach) zur Verfügung gestellt.

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Institut für Pharmakologie der Friedrich-Schiller-Universität Jena und Zoologische Station Neapel, den 1. Juni 1956.

Summary

δ-Tubocurarinchloride is shown to inhibit the respiration of sea-urchin eggs *in vitro* in concentrations up to 1·2 mg%. This effect is not inhibited by Prostigmin or Mestinon.

Quantitative Paper Chromatography of Lipids[1]

Paper chromatographic separations of lipids have been carried out mostly by using reversed phase methods with hydrocarbons as the stationary and polar solvents (methanol, acetic acid, water) as the mobile phase[2]. The commonly used indicators for staining have been Rhodamin B, copper acetate combined with potassium ferrous cyanide[3], or similar compounds which are restricted essentially to the detection of lipids having a free carboxyl group. Indicator methods independent of functional groups have been devised for unsaturated esters by chromatographing their mercuric acetate complexes[4] and detecting them with diphenylcarbazone which forms a colored spot with the mercury compound. All lipids chromatographed on glass fiber paper can be located by charring with sulfuric acid[5]. Use of paper chromatography for the quantitative determination of lipids has been reported only for one of these methods[6] and has been limited to the determination of acids.

Recently MANGOLD, LAMP and SCHLENK[7] have described the chromatography of lipids on siliconized paper as the stationary phase with aqueous acetic acid as the mobile phase. This system separates lipids consisting essentially of one long-chain moiety (acids, esters, aldehydes, alcohols, monoglycerides) while a chloroform-methanol mixture can be used to separate the triglycerides. Two indicators cover the whole variety of compounds, regardless of their functional groups. Iodine vapors react with any lipid having double bonds to yield yellowish-brown spots on a light background. Saturated lipids can be detected by means of α-cyclodextrin (cyclohexaamylose) and subsequent iodine treat-

[1] This work has been supported by a research grant from the National Institutes of Health (P. H. S. 4226) and by the Hormel Foundation. Hormel Institute publication No. 143.

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ment, as long as the lipid undergoes the inclusion reaction with α-cyclodextrin. The inclusion complexes can be formed on the paper after development of the lipid chromatogram by spraying it with α-cyclodextrin. When reacted with the lipid, α-cyclodextrin is inert to iodine vapors, while in free form it yields a purplish color instantaneously with iodine. Accordingly the lipids are detected as white spots on a purple background. Saturated di- and triglycerides do not react with cyclohexaamylose and cannot be located by this simple device.

	Analysis 1	Analysis 2	Analysis 3
	Acids %	Methyl esters %	Monoacid triglycerides %
Stearic	–	9·3 (10·0)	–
Oleic	30·7 (29·3)	27·5 (26·2)	20·3 (19·2)
Linoleic	23·2 (24·0)	33·4 (31·5)	46·5 (47·0)
Linolenic	46·5 (46·8)	29·8 (32·3)	35·4 (33·9)

These methods are applicable to the quantitative determination of lipids. Mixtures of fatty acids of the C_{18} series, of their methyl esters, or of the unsaturated mono-acid triglycerides have been analyzed by paper chromatography. Some results are given in the following compilation, where the theoretical values are listed in parentheses.

The procedures were essentially the same as previously reported. Siliconized paper (Dow Corning 200 fluid on Whatman No. 1 paper) was used in ascending chromatographic technique. The chromatograms of the fatty acids or esters were developed for 16–20 h at 30° ± 1° C with a mixture of aqueous 88% formic and 85% acetic acids (1:1 by volume) while the triglycerides were separated at 24° ± 1° C with a chloroform-methanol solvent mixture (3:1 by volume). A densitometer was used for measuring the optical densities of brown spots in blue light (filter 445 mμ) and for measuring the transmission of white spots on purple background in green light (filter 525 mμ). Model mixtures of known composition were chromatographed with an unknown on the same sheet, and the areas of the optical absorption curves were compared. After one or two adjustments of the component ratios in the model blends, linear interpolation is permissible for calculating the composition of the unknown.

The direct determination of oleic and stearic acids or their esters, which so far is possible only by paper chromatography, will often be advantageous. Higher unsaturated acids are commonly determined by alkaline isomerization. Notwithstanding merits of this latter method, the smaller amounts required for paper chromatography will make this technique very useful in many cases. To our knowledge, no method for the paper chromatographic separation and assay of triglycerides has been reported previously.

A more detailed report will appear in the near future.

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Hormel Institute, University of Minnesota, Austin, Minn., May 24, 1956.

Zusammenfassung

Ein qualitatives Verfahren zur papierchromatographischen Trennung von Fettsäuren und Derivaten liess sich zur quantitativen Bestimmung ausbauen. Die Methode hat eine Fehlergrenze von etwa ± 5% und ist unabhängig von den funktionellen Gruppen der Moleküle. Sie ist für gesättigte Di- und Triglyceride nicht direkt anwendbar.

The Influence of Albumin on the Electrophoretic Mobility of Serum Lipids *

MAURER[1] reported in 1953 that the addition of serum or albumin to the buffer profoundly altered the distribution of phospholipids in paper electropherograms of rat and rabbit sera doubly labelled with S^{35} and P^{32}. While he found almost all of the phospholipids associated with the serum globulins before addition of the "carrier protein", albumin appeared to form a complex with the phospholipids which migrated with the electrophoretic mobility of an albumin.

MAURER's experiments imply that the molecular structure of the lipoprotein complexes, which have been shown to contain most of the serum phospholipids and cholesterol[2] must be based on only very weak associations between the protein and lipid moieties, since albumin appeared to displace the protein component by a simple mass action effect. Such a dependence of lipoprotein structure upon the protein composition of the medium would invalidate much of the work of GOFMAN and PAGE on the determination of lipoprotein subfractions since these ultracentrifugal analyses were carried out in protein-poor solutions. While lipoproteins have long been considered labile structures and have been shown to exchange component lipids even *in vitro*[3] there is some evidence which argues against MAURER's views: It would be very difficult to explain the differences in solubility and electrophoretic mobility between α- and β-lipoproteins within the framework of MAURER's postulate. Moreover, RUHENSTROTH-BAUER[4] has demonstrated recently that the protein moiety of human serum lipoproteins is specific and cannot be replaced by either albumin or γ-globulin. In order to resolve this controversy we have reinvestigated the effect of added albumin on the electrophoretic mobility of serum phospholipid and cholesterol in human, hypercholesterolemic rabbit, and hypercholesterolemic rat sera. The effect upon phospholipid mobility alone was determined in normal rat and rabbit sera, where low cholesterol levels made analysis for this compound impractical.

In all experiments the sera were labelled biologically by administering radiophosphorus** to the donor 48 h before bleeding. Hypercholesterolemia was induced in rabbits by feeding a diet enriched with 1% cholesterol and 5% corn oil for 14 weeks, and in rats by the intra-

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[4] G. RUHENSTROTH-BAUER, Z. ges. exper. Med. *121*, 475 (1953).

** The Radioisotopes used in this investigation were supplied by the Oak Ridge National Laboratory on authorization from the Isotopes Division, U. S. Atomic Energy Commission.