Richard's medium for 8 weeks and sterilized by seitz filtration) prechelated with ferric chloride at the following levels of Fe''' for 24 h: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{5}–M. Fe''' was not toxic at levels lower than 10^{-3}M. and greatly increased the toxic symptoms as shrivelling, yellowing and vein clearing at the optimum level indicating that the active toxic principle of *F. vasinfectum* acts in a manner analogous to lycomarasmin on tomato by forming a chelate complex with Fe'''. Cystine was added to the chelate complex (at 10^{-3}M. Fe''' level) at the following levels both before and after chelation: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}M. Cystine was not toxic to the cut shoots at levels lower than 10^{-4}M. nor was the cystine-iron complex. Addition of cystine at the optimum level 10^{-4}M. to the toxin-iron complex before chelation gave effective protection to the cut shoots which indicated no toxic symptoms compared to the controls without cystine. A complete set of controls was maintained. Cut shoots pretreated to cystine at the optimum level for 24 h were able to overcome the toxic effects of the toxin-Fe''' complex. In the absence of added Fe''' cystine produced no significant beneficial effects in overcoming the toxicity. The results suggest that cystine present in the resistant varieties of cotton possibly chelates with the Fe''' available in the host and renders it unavailable for chelation with the toxin which seems to be a pre-requisite for toxigenic wilting of cotton. The availability of free ferric iron in the hosts for the *in vivo* formation of the toxic chelate would thus be an important factor in determining resistance or susceptibility to wilt.

Further work on the mechanism of cystine formation and its chelation is underway and is likely to throw more light on the mechanism of wilt resistance in cotton.

It is a pleasure to record my indebtedness to Professor T. S. SADASIVAN for guidance and to Dr. C. V. SUBRAMANIAN for his keen interest in the work. My thanks are also due to the Government of India for the award of a senior research scholarship.

K. LAKSHMINARAYANAN

University Botany Laboratory, Madras-5, India, June 30, 1955.

Zusammenfassung

Cystin verhindert *in vitro* in Gegenwart von Fe^{III}-Ionen bei sensiblen, abgeschnittenen Baumwollschösslingen eine Vergiftung durch Kulturfiltrat von *Fusarium vasinfectum*. Die mögliche Beteiligung von Cystin am Mechanismus der Welkeresistenz von Baumwolle wird diskutiert.

The Precipitation of Cytochrome C with a Lipidic Fraction from Tissues and Yeast

During some research work on the influence of lipidic extracts from baker yeast on the succinoxidase activity of homogenates and mitochondria from rat liver, it was observed that precipitate was formed almost immediately after the addition of cytochrome C. It seemed most likely that this precipitate contained the cytochrome C.

The system adapted in the experiment was: 0·20 ml of rat liver homogenate prepared in Potter-Elvehjem and diluted 1:10 with 0·25 M sucrose + 2 ml of 0·067 M Puffer Phosphate Ph 7·4 + 0·20 ml of $AlCl_3$ + 0·20 ml of $CaCl_2$ + 0·40 ml of Na succinate (final concentration: 0·125 M) + 1 mg of cytochrome C. Gas: O_2.

Following the above observation, I investigated the reason for this precipitation.

Material and Methods

The lipidic fraction from baker's yeast, and from rabbit's or rat's liver was prepared according to FAURE's technique[1]; a slight modification was introduced: 700 ml of sulphuric ether was added to 0·5 kg of baker yeast and shaken; then, 700 ml of 95% alcool was also added and shaken again; the material was then placed in water-bath at 37° for 12 h and then dried in a crystallizator at a temperature of 37°.

The dried material was treated with 100 ml of $CHCl_3$ at room temperature; the insoluble residue which consisted mainly of polysaccharides was then discarded by filtration. The filtrate was treated with 2 volumes of acetone, the resulting precipitate was separated by centrifugation at low speed, and then treated with ether. The fraction of precipitate which was soluble in ether contained mainly lecithines, cephaline and phosphatides (fraction I), whilst the insoluble fraction (fraction II) contained mainly cerebrosides and sphyngomyeline.

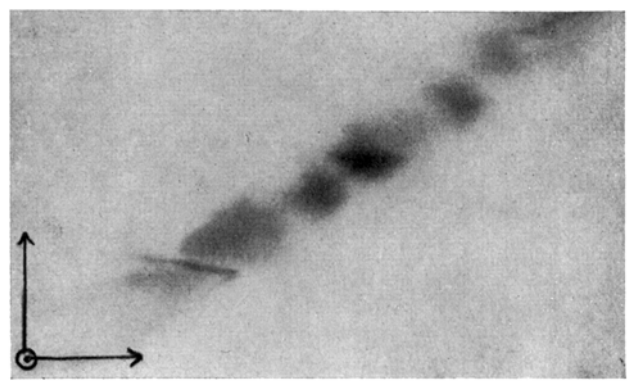

Fig. 1. – From right to left: Phenylalanine, leucine, alanine, threonine, glutamic acid, glycine, serine, aspartic acid, histidine, exonic basis.

Fraction I was the part which interested the investigator and was subjected to the following analyses:

(1) Paper electrophoresis (which was made with an Elphor apparatus): no migrating protein was detected.

(2) Paper chromatography: no free amino acids were found. After hydrolysis with 6 NHCl for 24 h and desalting with Consden apparatus, 6–10 amino acids (see Fig. 1) became evident.

Two preparations of cytochrome C were used: the first one was prepared from horse heart according to the method of KEILIN and HARTREE and contained 0·35% Fe; the second one was an electrophoretically purified preparation which was received through the courtesy of I.S.M.; its Fe content was 0·43%.

In a first experiment, 0·10 ml of a 10% suspension of lecithines prepared from baker yeast were added to a 1% solution of cytochrome C in distilled water. Almost immediately a dense red coloured precipitate was formed. The precipitate was separated by centrifugation. Nitrogen and P contents of the original lecithine emulsion and of the original cytochrome C solution as well as of both precipitate and supernatant fluid were determined. The results obtained are summarized in the Table:

[1] M. FAURE, *Techniques de Laboratoire* (Masson Publ., Paris 1947).

	Nitrogen mg	Phosphorus μg
Cytochrome solution	1·428	6·90
Lecithine emulsion (in 0·10 ml) . .	0·154	15·8
Precipitate	0·756	16·22
Supernatant	0·840	6·48

It is clear from the Table that a large part of cytochrome C is precipitated from the solution by the addition of lecithine emulsion. The precipitate was insoluble in acetone, but was soluble in ether; the colour of the solution was red. The absorption curve of this solution is shown in Figure 2. It is clear that the curve shows a striking difference from that of cytochrome C in the regions between 300 and 400 mμ, and between 500–560 mμ. Lecithine material from different sources was also treated for its ability to precipitate cytochrome C. Lecithine extracted from rat or rabbit livers was as active as that from baker yeast. Lecithine prepared from chicken eggs produced, on the contrary, only a very faint precipitate when added to cytochrome C solutions.

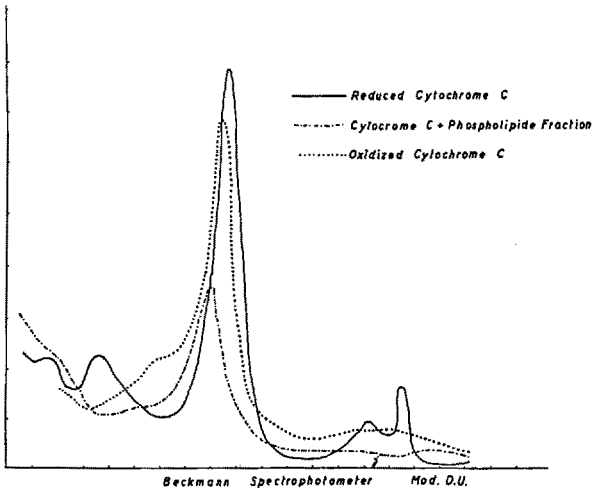

Fig. 2.

The digestion of yeast or liver lecithine fraction with 5 mg Clostridium Welchii lecithinase (20 min at 38°C) did not modify their ability to precipitate cytochrome C. Also tryptic and papainic digestions were ineffective.

Treatment of fraction I with saturated $(NH_4)_2SO_4$ produced the formation of a dense conglomerate on the surface of the fluid. This material retained its ability to precipitate cytochrome C after dialysis against distilled water at 0°C for 48 h. Heating at 100°C for 20 min did not modify the precipitating power. The addition of a small amount of the lecithine emulsion to an albumine solution from chicken eggs or to human haemoglobin dissolved in distilled water did not produce any precipitation. A strong precipitate was formed, however, when fraction I was added to a solution of iron salt such as $FeSO_4$ or Ferric ammoniacal citrate.

Modifications of the biological properties of cytochrome C: In this experiment the reaction system contained 0·3 ml of a 10% suspension of rat liver mitochondria in 0·25 M sucrose, 6 mg of Na ascorbate, 2 mg cytochrome C, 0·2 ml 30% KOH in the central well. Gaseous environment was 100% O_2.

In some instances, 0·1 ml of fraction I was added. In these cases, the precipitate was rapidly formed and the O_2 uptake was nil. In another experiment, the amount of cytochrome C was 10 mg instead of 2 mg. This represented a large excess with respect to the amount of fraction I which was added. The aim of this experiment was to see if cytochrome C which remained in solution after treatment with the lecithine fraction maintained its biological activity. The O_2 uptake observed in this experiment was 52·7 l, while it was 49·5 l in the system in which fraction I was not added, this means that the biological activity was not lost by cytochrome C remaining in solution.

L. MICHELAZZI

Department of General Pathology, University of Genoa, June 16, 1955.

Riassunto

Nel corso di ricerche sopra l'azione esplicata da alcune frazioni lipidiche sulla succinossidasi presente nei mitocondri di fegato di ratto, l'autore ha osservato che il citocromo C aggiunto al sistema precipita in presenza della frazione contenente lecitine cefaline e fosfatidi, estratta dal lievito o dal fegato di animali diversi.

Di questo materiale è stata compiuta l'analisi elettroforetica su carta che non ha rivelato proteine migranti. L'analisi cromatografica su carta non ha mostrato aminoacidi liberi, ma dopo idrolisi 6–10 aminoacidi sono evidenziabili. Il precipitato risultante dalla unione della frazione lecitinica con il citocromo C è stato analizzato per il suo contenuto in P ed in N comparativamente ai materiali di origine. Detto precipitato è solubile solo in etere e la curva spettrofotometrica del citocromo C in queste condizioni appare modificata rispetto alla norma.

La frazione lecitine non precipita in presenza di albumina di uovo e di emoglobina umana, mentre dà un forte precipitato in presenza di solfato di Fe e di citrato ferrico ammoniacale.

L'attività biologica del citocromo C si annulla completamente con la precipitazione.

Isolierung fluoreszierender Stoffe aus *Astacus fluviatilis*

Bei *Drosophila melanogaster* konnte mit papierchromatographischen Methoden das Vorkommen zahlreicher fluoreszierender Stoffe und deren mengenmässige Abhängigkeit von Erbfaktoren nachgewiesen werden[1]. Ähnliche Untersuchungen wurden an *Ephestia kühniella*[2] und *Bombyx mori*[3] durchgeführt. Neuerdings ist es nun auch gelungen, die chemische Natur einiger dieser Substanzen aufzuklären[4].

[1] E. HADORN und H. K. MITCHELL, Proc. Nat. Acad. Sci. *37*, 650 (1951). – E. HADORN, Arch. Julius-Klaus-Stiftung *26*, 470 (1951). – S. NAWA und T. TAIRA, Proc. Japan. Acad. *30*, 632 (1954). – E. HADORN, Exper. *10*, 483 (1954).

[2] E. HADORN und A. KÜHN, Z. Naturf. *8b*, 582 (1953).

[3] S. NAWA und T. TAIRA, Proc. Japan. Acad. *30*, 632 (1954). – S. NAWA, M. GOTO, S. MATSUURA, H. KAKIZAWA und Y. HIRATA, J. Biochem. (Japan) *41*, 657 (1954). – R. TSCHESCHE, Angew. Chemie *66*, 302 (1954).

[4] S. NAWA und T. TAIRA, Proc. Japan. Acad. *30*, 632 (1954). – S. NAWA, M. GOTO, S. MATSUURA, H. KAKIZAWA und Y. HIRATA J. Biochem. (Japan) *41*, 657 (1954). – R. TSCHESCHE, Angew. Chemie *66*, 302 (1954). – H. S. FORREST und H. K. MITCHELL, J. Amer. chem. Soc. *76*, 5656 (1954). – M. VISCONTINI, M. SCHOELLER, E. LOESER, P KARRER und E. HADORN, Helv. Chim. Acta *38*, 397 (1955). – M. VISCONTINI, E. LOESER, P. KARRER und E. HADORN, Helv. Chim. Acta *38*, 1222 (1955).