of *C. capitata* which might be helpful in fruit fly research when planning a release programme against this insect pest, since most release projects depend on pupal distribution.

Time of emergence. An experiment was conducted to find out the time of day at which adult emergence took place in the laboratory at 25 °C and 60–65% RH. A set of 500 pupae were placed in a cylindrical jar on the expected day of emergence. Emerged adults were collected, counted and sexed at intervals of 2 h, starting from 05.00 h till 13.00 h. The results are shown in table 1.

A few adults emerged on the proceeding and preceeding days which were ignored. However, as shown above, most of emergence took place in the morning between 06.00 and 09.00 h. This finding would be of special interest for choosing the time of release of irradiated pupae, especially when bearing in mind the tremendous loss in released pupae due to the effect of climatic factors and to attack by ants and other soil insects when left on the ground for long time.

Depths at which adults emerge. Sets of 50 pupae, 2 days before the expected time of emergence, were placed in glass tubes 3 cm in diameter and covered with sand to heights of 1, 5, 10, 15, 20, 30 and 40 cm. Adults were found to emerge successfully without any significant differences from the controls till a depth of 30 cm, while at 40 cm none of the adults succeeded in emerging.

Weight of the pupae. An experiment was carried out to investigate the possibility of sexing the Medfly pupae according to their weight. For this purpose 3 sets each of 100 pupae, 2 days old, chosen at random, were weighed individually and allowed to emerge separately for sex determination. Results are shown in table 2.

Although results indicated a significant difference between the mean male and female weight of pupae ($p \leqslant 0.05$), the differences cannot be practically used by any laboratory technique for sexing. It was also observed that both overweight or underweight pupae did not emerge. It is clear that the pupal weight depends on the breeding media and technique, yet even with standard constant rearing conditions, it was difficult to differentiate between sexes by means of pupal weight.

Effect of tagging on pupal emergence. Fluorescent pigments are commonly used at present in ecological tagging studies, e.g. in dispersal, flight range and population size determination studies. Tagging of adults is achieved by applying the pigment to the pupae shortly before emergence[1]. For studying the effect of tagging on the percentage of emergence, an adequate number of pupae were collected on the same day of pupation, and 3 sets of 100 pupae each were drawn daily throughout the 10 days of the pupal stage and tagged with the fluorescent pigment at the same rate used for release experiments[1]. Results of adult emergence showed that the percentage of emergence was not affected by the time of tagging, except in pupae tagged on the 1st day where slight insignificant reduction in adult emergence was observed.

Table 1

Time	05.00	07.00	09.00	11.00	13.00	Totals
Males	-	110	78	12	-	200
Females	-	104	81	11	-	196
Totals	-	214	159	23	-	396

Table 2

Sex	Weight in mg		
	Maximum	Minimum	Mean ± SE
Males	9.7	6.1	8.1447 ± 0.16
Females	9.7	6.7	8.0733 ± 0.15
Non-emerged	12.5	3.0	6.2625 ± 0.37

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Effect of solubilization of *Salmonella minnesota* Re glycolipid on its interferon-inducing activity

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Summary. Electrodialysis of *Salmonella minnesota* Re glycolipid, or exposure to 0.2 M EDTA, pH 7.0, yields products capable of eliciting interferon production at concentrations 10-fold lower than that of the original glycolipid.

The elegant studies of Feingold et al.[1] have led to the recognition of the lipid A moiety of lipopolysaccharides (LPS) of Enterobacteriaceae as a major determinant of these potent interferon inducers. It has been conclusively proved[2] that *S. minnesota* Re glycolipid subjected to mild alkaline hydrolysis exhibits enhanced interferon-inducing activity as a result of the increased solubility of the altered molecule.

The studies of Galanos and Lüderitz[3] suggest that the state of aggregation of LPS contributes substantially to the expression of their biological activity. An electrodialysis procedure[4] has been elaborated for the removal of metal cations and basic amines from LPS, and the resulting derivatives have been found to possess remarkable solubility in water.

Taking cognizance of these latter findings, we decided to explore the effect of solubilization on the interferon-inducing capacity of the glycolipid derived from Re mutant of *S. minnesota*. To achieve this we tried 2 procedures of solubilization: a) electrodialysis and b) treatment with 0.2 M EDTA at pH 7.0.

Glycolipid (GL) was prepared from acetone-dried bacterial cells of *S. minnesota* Re by extraction with chloroform-methanol as outlined by Chen et al.[6]. The purity of the GL was verified by immunodiffusion and immunoelectrophoresis employing antiserum against the Re mutant of *S. min-*

nesota. The presence of a single precipitation line was indicative of the purity of the product. Furthermore the determination of the content of 2-keto-3-deoxyoctonate (KDO)[5] gave values which were close to those reported by Youngner et al.[2] for purified *S. minnesota* Re glycolipid.

The purified GL was then subjected to electrodialysis according to Galanos and Lüderitz[4]. After the completion of the procedure, the pH of the solution was brought to neutrality by adding triethylamine. EDTA treatment of GL was carried out essentially as described by Rietschel et al.[7]. Prior to interferon induction, the concentration of the treated GL was precisely measured by KDO determination[5].

Interferon induction was tested in explanted mouse peritoneal leucocytes[8]. The cell suspension (2.5×10^6 cells/ml) was treated with the GL at concentrations ranging from 0.01 ng to 10^4 ng/ml and the incubation was allowed to proceed at 26 °C[9,10]. Samples were taken at the 20th h[11]. Interferon production was assayed in L-cells challenged with vesicular stomatitis virus.

A dose-response curve is presented in the figure. From it may be concluded that:

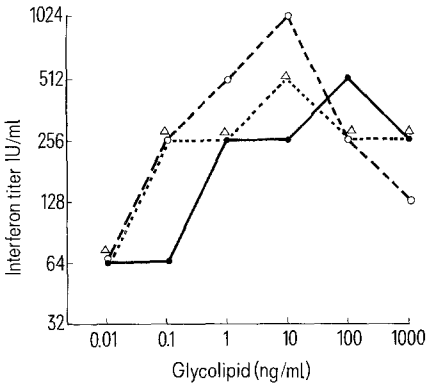
1. The minimal interferon-inducing concentration of the electrodialyzed and EDTA-treated GL comprised $^1/_{10}$ of the concentration of the original GL (0.1 ng vs 1.0 ng).
2. Both GL derivatives elicited peak levels of interferon (optimal interferon-inducing concentration) at concentrations which were 10-fold lower than that of the GL (10 ng and 100 ng, respectively).

When the interferon titer was plotted against the corresponding doses of the GL and its derivatives, a dose-response curve typical for endotoxins was obtained (figure). It should be emphasized that at higher GL concentrations the interferon-inducing activity tended to decrease, which is probably due to cytotoxicity[11].

In addition, it should be mentioned that the ratio of the optimal/minimal interferon-inducing concentration was approximately 100 for the 3 products examined.

The 10-fold increase of the interferon-inducing capacity of the electrodialyzed and EDTA-treated GL may be ascribed to the increased solubility of these 2 products.

Our results suggest that the solubility of the GL of *S. minnesota* Re exerts a marked effect on LPS-mediated interferon induction. This agrees favorably with the data reported by Youngner et al.[2] that the solubilization of GL resulting from partial alkaline hydrolysis or complexing with bovine serum albumine leads to enhanced interferon production.



Salmonella minnesota Re glycolipid-induced interferon production in mouse peritoneal leucocytes. ●——● Glycolipid, O– – –O electrodialyzed glycolipid, △·····△ glycolipid treated with 0.2 M EDTA, pH 7.0.

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Specific inhibition of formation of acid-fastness in mycobacteria by 3,3′-di-O-methylellagic acid

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Summary. 3,3′-Di-O-methylellagic acid obtained from *Euphorbia adenochlora* selectively inhibited the formation of acid-fastness in mycobacteria without retardation of their growth. Gross reductions in contents of wax D, cord factor and free mycolic acids were found in the nonacid-fast bacilli compared with the normal ones.

In general, acid-fastness of mycobacteria has been understood through the characteristic property of the outer cellular materials to form acid-stable complexes with aminoarylmethane dyes. Several investigators have reported that the mycolate residues were responsible for the acid-fastness of mycobacteria[1,2]. However, Murohashi et al. showed no correlation between amount of mycolic acids and strength of the acid-fastness[3]. On the other hand, another group reported that young mycobacteria revealed nonacid-fastness and exhibited resistance to degradation with lysozyme[4], glycine[4] and some chemical mutagens[5], and the mycobacterial acid-fastness might, therefore, be associated with the integrity of cell-walls.

As the first aim of our work was to investigate the mechanism of acid-fastness, we performed experiments to inhibit specifically the formation of acid-fastness in mycobacteria. We now wish to report that nonacid-fast mycobacteria were successfully obtained from acid-fast cultures after the application of the naturally occurring substance, 3,3′-di-O-methylellagic acid. Although the occurrence of inhibitors