observations is the 'dynamic membrane flow hypothesis'[21,22] which suggests the interconversion of phospholipids between different cellular membrane systems and organelles. This can be further emphasized by the postulated role of phospholipids as carriers of cations and other substances across the cell membrane[23].

1 Acknowledgments. N.P. thanks the Council of Scientific and Industrial Research, India for the award of Senior Research Fellowship.
2 To whom reprint requests should be addressed.
3 Verma, J.N., and Khuller, G.K., Adv. Lipid Res. (1983) in press.
4 Khuller, G.K., and Subrahmanyam, D., Immunochemistry *8* (1971) 251.
5 Trana, A.K., and Khuller, G.K., Indian J. med. Res. *67* (1978) 734.
6 Goldman, D.S., Am. Rev. Res. Dis. *102* (1970) 543.
7 Ballou, C.E., Meth. Enzym. *28* (1972) 493.
8 Taneja, R., and Khuller, G.K., Archs Microbiol. *129* (1981) 81.
9 Verma, J.N., and Khuller, G.K., FEMS Microbiol. Lett. *11* (1981) 55.
10 Banerjee, B., Jain, S.K., and Subrahmanyam, D., J. Chromat. *94* (1974) 342.
11 Kearney, E.B., and Goldman, D.S., Biochim. biophys. Acta *197* (1970) 197.
12 Grover, G., Dhariwal, K.R., and Venkitasubramanian, T.A., J. gen. Microbiol. *105* (1978) 343.
13 Akamatsu, Y., Ono, Y., and Nojima, S., J. Biochem. *59* (1966) 176.
14 Oka, S. Kukushi, K., Fujimoto, M., Sato, M., and Motomiya, M., Crit. Rev. Soc. Biol. *162* (1968) 1648.
15 Akamatsu, Y., Ono, Y., and Nojima, S., J. Biochem. *61* (1967) 96.
16 Khuller, G.K., Banerjee, B., Sharma, B.V.S., and Subrahmanyam, D., Indian J. Biochem. Biophys. *9* (1972) 274.
17 Hackett, J.A., and Brennan, P.J., Biochem. J., *148* (1975) 253.
18 Talwar, P., and Khuller, G.K., Indian J. Biochem. Biophys. *14* (1977) 72.
19 Khuller, G.K., and Trana, A.K., Experientia *33* (1977) 1422.
20 Barksdale, L., and Kim, K.S., Bact. Rev. *14* (1977) 217.
21 Nozawa, Y., and Thompson, Jr, G.A., J. Cell Biol. *49* (1971) 722.
22 Jalsema, C.L., and Noore, J., J. biol. Chem. *253* (1978) 7960.
23 Fourcans, B., and Jain, M.K., Adv. Lipid Res. *12* (1974) 147.

0014-4754/83/080882-03$1.50 + 0.20/0

The effect of diet on esterase band pattern in *Myzus persicae* (Sulzer) – a disclaimer

T.C.R. White[1]

School of Applied Science, Riverina College of Advanced Education, Wagga Wagga, N.S.W. (Australia), December 22, 1982

Summary. Aphids cultured on artificial diet did not exhibit extra esterase band patterns, nor did their patterns differ from those in aphids cultured on plants.

Bunting and van Emden[2] reported that polyacrylamide gel electrophoretograms of aphids from the insecticide-susceptible Y5 strain of *Myzus persicae* cultured for over 2 years on Dadd and Mittler's[3] artificial diet exhibited a number of hitherto unreported esterase band patterns; and these bands were not present in gels made from aphids taken from this clone and reared for several generations on potato plants. While discussing a number of possible explanations for this phenomenon and concluding that the final answer must await further work, they stated that their work '... demonstrates beyond doubt that diet can have a profound effect on esterase band pattern'.[2] (My emphasis).

Materials and methods. As a prelude to investigating further this reported influence of diet on changes in band patterns of esterases of *M. persicae,* I set out to provide the conventional repetition of original results by running several preliminary gels of bulk homogenates of aphids taken from this same Y5 clone. I used the same materials and methods as those used by Bunting and van Emden[2], conducted the work in the same laboratory, using the same equipment, and, as a control, ran, on the same gels, homogenates of aphids from brussel sprout plants growing in the Department greenhouse. The only known difference was that the Y5 clone had now been maintained on the artificial diet for more than 5 years without interruption.

Results. These first runs produced gels which showed no difference in esterase band patterns between the aphids grown on the artificial diet and those from the brussel sprout plants. And neither population produced gels showing any sign of the extra esterase bands reported by Bunting and van Emden[2]. I then ran further gels contrasting alate and apterous adults, nymphs and adults, populations from different glasshouses and different host plants; and using concentrations of homogenate from 60 µg aphid/µl to 120 µg aphid/µl. I varied the voltage and times for running the gels and the times for staining the gels.

I also ran gels of aphids taken from every sachet of artificial diet on which the Y5 clone was being maintained, from Y5 individuals parasitized in the laboratory by *Aphidius matricariae,* from Y5 populations transferred to plants and back to artificial diet, from sachets of Y5 populations kept for 4 weeks in a refrigerator at approximately 5 °C, and using homogenates frozen for up to 72 h prior to use.

None of these gels produced any sign of the extra esterase bands. I then contacted Bunting and discussed his techniques in some detail. As a result I made several minor adjustments to the method of preparing solutions and of preparing and running the gels. But further runs still failed to produce gels showing any sign of extra bands.

Conclusions and discussions. It can be assumed with a fair degree of confidence that the present Y5 clone of *M. persicae* no longer includes aphids which produce polyacrylamide gels showing extra esterase bands, or patterns of bands which differ in any significant way from those of aphids feeding on plants.

A number of explanations could be proposed to account for this failure to repeat Bunting and van Emden's[2] findings. But Bunting (personal communication) states that gels illustrating these bands in the original Y5 population were never made prior to establishing the sub-population from that clone onto potato plants. Nor were the 2 sub-populations from which his definitive gels were finally made

derived from a single individual, nor even from only one of the several sachets of artificial diet on which the original Y5 population was being cultured. And he made only 2 gels contrasting the 2 populations. There is, therefore, no evidence that these extra bands were ever present in the original population of the Y5 clone, or if they were, that they were not confined to one or a few aberrant individuals from which the sub-culture on artificial diet (but not that on potato plants) was derived.

There could be many other explanations for the appearance of these extra bands in only 1 of these 2 sub-cultures, and for their subsequent complete disappearance. But my repeated failure to find any trace of these extra bands in gels made from aphids of the same Y5 clone, and the failure of the original authors to demonstrate the presence of these bands before subjecting the sub-cultures to different diets, precludes any claims being made that diet has an effect on the pattern and number of the esterase bands of *M. persicae.*

1 I thank Professor H.F. van Emden for the opportunity to work in his laboratory and Josephine Pemberton and Barry Tranter for their guidance, help and friendship throughout my apprenticeship in acrylamide-gel-electrophoresis.

2 Bunting, S., and van Emden, H.F., Experientia *37* (1981) 220.
3 Dadd, R.H., and Mittler, T.E., Experientia *22* (1966) 832.

0014-4754/83/080884-02$1.50 + 0.20/0

Purification of diphtheria toxin by chromatography on Cibacron Blue-Sepharose

G. Antoni, M. Bigio, G. Borri, M.C. Casagli and P. Neri

ISVT Sclavo Research Center, via Fiorentina 1, I-53100 Siena (Italy), December 13, 1982

Summary. Diphtheria toxin binds to Cibacron Blue-Agarose and may be eluted by increasing the ionic strength of the elution buffer. Experiments using difference spectroscopy showed that the interaction between toxin and dye is ionic rather than hydrophobic, and therefore it is of a different nature with respect to that usually found in nucleotide-requiring enzymes.

Diphtheria toxin inhibits protein synthesis in mammalian cells, catalyzing ADP-ribosylation of elongation factor 2[1]. The toxin exerts its toxic activity after formation of a 1:1 noncovalent complex with NAD[1,2]. Exploiting this interaction, the toxin has been purified by affinity chromatography on NAD-Sepharose[3].

Enzymes which interact with NAD generally also bind the dye Cibacron Blue F3GA, and may be purified by chromatography on a column of Cibacron Blue-Sepharose[4]. The interaction with the dye has been adopted as a probe for the protein supersecondary structure called the 'dinucleotide fold'[4], although the binding is not completely specific, and proteins devoid of the dinucleotide fold may also interact with the dye[5]. It was therefore of interest to examine whether there was also an interaction with Cibacron Blue in the case of diphtheria toxin, which would in some way reflect the interaction with NAD.

We performed our experiments using a crude broth culture of *C. diphtheriae,* grown on a Lingood medium. After removal of the bacteria by centrifugation, the supernatant was sterilized by filtration and dialyzed against 0.02 M phosphate buffer pH 6.9. The solution was then chromatographed on a column of Blue Sepharose CL-6B (Pharmacia, Sweden), giving the elution profile shown in figure 1. The 1st peak of UV-absorbing material was obtained by eluting the column with 0.02 M phosphate buffer, pH 6.9. The buffer was then changed to one containing 2M NaCl, which gave a 2nd peak containing most of the immunochemical activity. The combined fractions of the 2nd peak showed a specific immunochemical activity of about

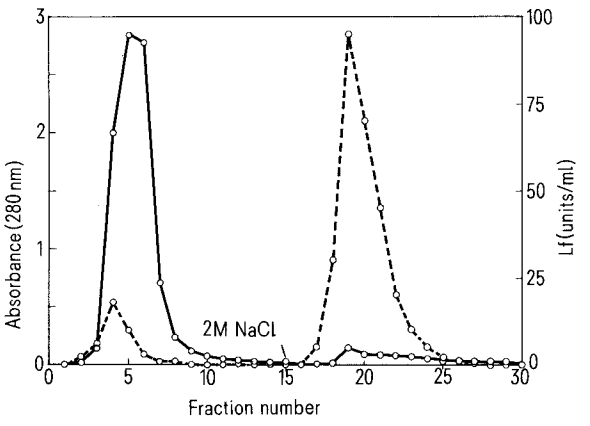

Figure 1. Chromatography of crude diphtheria toxin on a column of Cibacron Blue-Sepharose (1.6×20 cm). Volume of the sample: 30 ml (200 Lf/ml). Volume of the fractions: 10 ml. Flow rate: 70 ml/h. The protein content of the fractions was estimated by measuring the absorbance at 280 nm. The immunochemical activity (dotted line) was determined by rocket immunoelectrophoresis[6] against a specific antiserum, using a reference preparation of toxin, 75 Lf/ml, as standard.

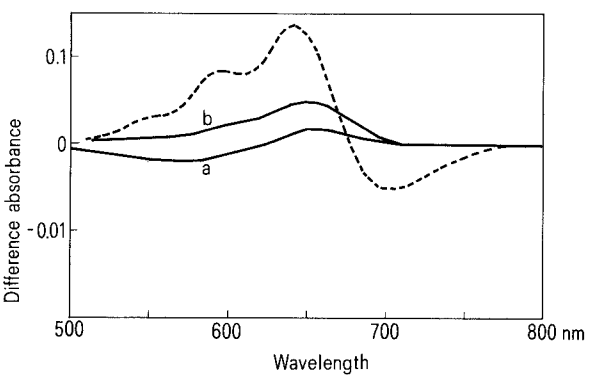

Figure 2. Difference spectrum of Cibacron Blue with diphtheria toxin (highly purified reference sample). The sample cuvette contained 15 μM toxin and Cibacron Blue at concentrations: a) 135 μM; b) 260 μM. The reference cuvette contained the same amount of dye as the sample cuvette. The dotted line presents the spectrum obtained using 15 μM human serum albumin in place of diphtheria toxin and 135 μM Cibacron Blue.