expected 2 bands. Evidence for lack of post-meiotic gene expression is also reported for glutamate oxalacetic transaminase[4]. Pre-meiotic synthesis of β-glucuronidase with persistence in post-meiotic stages has been suggested in rat testis[5]. However, as I have previously argued[6], the presence of intercellular bridges between spermatids provides a syncytial quality to the developing spermatozoa and would help to assure gamete equivalence.

Another conclusion this work provides is that the structural gene for spermatozoal β-glucuronidase is the Gus locus. The evidence for this comes from the demonstration by electrophoresis of the expression of the Gus^a and Gus^b alleles. This conclusion is interesting in that certain enzymes have been shown to have a sperm-specific isoenzyme (e.g. lactate dehydrogenase, hyaluronidase). A third conclusion is that spermatozoal β-glucuronidase shows the electrophoretic properties of the lysosomal form. This is not surprising as it is the lysosomal form which does not have attached peptides, but the finding is also relevant to the question of acrosome formation. The acrosome is known to contain many lysosomal enzymes and has been postulated to be a specialized lysosome[7], while the fact that acrosomal hyaluronidase and proteolytic activities are very different from those of lysosomes suggests less of an homology of acrosomes to lysosomes. If Mathur[8] is correct in asserting that β-glucuronidase is found in the mid-piece, then this lack of localization of lysosomal enzyme in the acrosome would argue further for the uniqueness of the acrosome in contrast to lysosomes.

4 R. P. Erickson, Exp. Cell Res. *86*, 429 (1974).
5 J. L. Males and R. W. Turkington, Endocrinology *88*, 579 (1971).
6 R. P. Erickson, Nature New Biol. *243*, 210 (1973).
7 A. G. Allison and E. F. Hartree, J. Reprod. Fert. *21*, 501 (1974).
8 R. S. Mathur, J. Reprod. Fert. *27*, 5 (1971).

Evidence of the involvement of membrane-bound steroids in the photoperiodic induction of flowering in Xanthium

B. Mitra and S. P. Sen[1]

Botany Department, Kalyani University, Kalyani 741235 (India), 8 June 1976

Summary. Filipin, which interferes with sterol stabilization of phospholipid membrane layers, inhibits the photoperiodic induction of flowering in Xantium; the inhibition is reversed to a large extent by cholesterol.

The role of steroidal substances in plant flowering is a matter of controversy[2,3]. Application of oestrogenic substances, phytosterols and several other steroids have been reported to promote flowering of several plants and increased levels of oestrogen, oestrogen-like substances and sapogenins have been observed at the time of flowering or under photoinductive conditions. The relevant recent literature has been reviewed by Grunwald[4]. We report here our observations which suggest the involvement of membrane-bound steroids in flowering.

The polyene antibiotic filipin interacts specifically with sterols and inhibits the growth of those organisms which have membrane-bound steroids. Thus, it inhibits the growth of fungi and the inhibition is countered by sterols[5]; but species of Pythium which lacks sterol is completely unaffected by filipin and sterol treatment results in the conferment of sensitivity to filipin. Essentially similar results were obtained with mycoplasma laidlawii, which also lacks membrane-bound steroids[6]. Filipin also mediates permeability changes in erythrocytes[7]. Interaction of filipin with natural and synthetic membranes containing cholesterol has elucidated the mechanism of the interaction. The permeability effects are reversible with cholesterol[8,9].

Xanthium brittoni plants can be induced to flower with three photoinductive cycles. Photoperiodic effects on Xanthium buds are quantitative and bud growth from the vegetative condition to the development of influorescence primordium can be separated into 8 distinct stages numbered in an ascending order of development. Salisbury[10] has shown that the rate of floral bud growth can be most conveniently assessed from the stage number to which the developing floral bud belongs at any given time. The average stage corresponding to a particular treatment reflects the average advancement towards reproductive maturity. Filipin was applied on apical and the next 5 axillary buds of Xanthium at a concentration

Effect of filipin on photoperiodic induction of Xanthium and its reversal by cholesterol

Bud	Average of bud stages* Control	Filipin (100 μg/ml)	Filipin (100 μg/ml) + cholesterol (50 μg/ml)
Apical	8.00	6.00±0.30**	8.00
Axillary 1	7.67±0.19	3.50±0.34**	7.25±0.29
Axillary 2	5.33±0.20	2.37±0.30**	5.00±0.30
Axillary 3	5.00±0.20	1.00±0.20**	2.17±0.28**
Axillary 4	2.19±0.29	–	1.25±0.21***
Axillary 5	1.20±0.33	–	–

Each treatment was applied before each of 3 photoperiods. Scored after 10 days. * Bud stages scored according to Salisbury[10]. ** Significant at P = 0.01. *** Significant at P = 0.05.

1 We are thankful to Upjohn Co., Kalamazoo, Mich., USA, for the gift of a sample of filipin used in this investigation.
2 E. Heftman, A. Rev. Pl. Physiol. *14*, 225 (1963).
3 L. T. Evans, A. Rev. Pl. Physiol. *22*, 265 (1971).
4 C. Grunwald, A. Rev. Pl. Physiol. *26*, 209 (1975).
5 D. Gottlieb, J. H. Cautcy, L. C. Wu und J. H. Sloneker, Phytopathology *50*, 594 (1960).
6 M. M. Weber and S. C. Kinsky, J. Bacteriol. *89*, 306 (1965).
7 S. C. Kinsky, Archs Biochem. Biophys. *102*, 180 (1963).
8 J. O. Lampen, P. M. Arrow and R. S. Shafferman, J. Bacteriol. *80*, 200 (1960).
9 A. W. Norman, R. A. Demel, B. D. Kruyff and L. L. M. Van Deenen, J. biol. Chem. *247*, 1918 (1972).
10 F. B. Salisbury, Pl. Physiol. *30*, 327 (1955).

of 100 µg/ml, just before each of three 16 h photoperiods and the advancement of the buds towards reproductive condition was scored according to Salisbury[9] by examination of the buds under a light microscope after 10 days. The table shows that filipin at the concentration used has severe inhibitory effects on the flowering of Xanthium. Since in animal systems and in acholeplasma laidlawi the inhibitory effect of filipin is reversed by cholesterol, we examined the effect of cholesterol in relation to filipin action. It was observed that the filipin effect could not be demonstrated in buds pretreated with cholesterol (50 µg/ml), particularly in the lower axillary buds. Higher concentrations of cholesterol could not be used due to difficulty in dissolving cholesterol in aqueous alcoholic solutions. In Xanthium, photoperiodic effects on the axillary buds decrease with increase in distance from the shoot apex.

Photoperiodic induction thus, in some way involves the participation of steroids, presumably membrane-bound ones. That filipin affects permeability of plant systems has been suggested by Hendrik and Higinbotham[11] for pea mitochondria, by Bishop[12] for pea and maize chloroplasts, by Grunwald[13] and Mudd and Kleinschmidt[14] for sugar beet root discs and by Das[15] for wheat coleoptiles. It is now becoming increasingly evident that several phytosterols are associated with plant membranes[4]. It is interesting to mention here that the pigment phytochrome, which controls flowering in plants, is also associated with plant membranes, and many workers believe that phytochrome effects are mediated through a control of membrane permeability. An understanding of the role of membrane-bound steroids in plant flowering will depend on the precise location of such steroids and phytochromes in plant membranes.

11 D. L. Hendrik and N. Higinbotham, Pl. Physiol. *52*, 93 (1973).
12 D. G. Bishop, Archs Biochem. Biophys. *154*, 520 (1973).
13 C. Grunwald, Pl. Physiol. *43*, 484 (1968).
14 J. B. Mudd and M. G. Kleinschmidt, Pl. Physiol. *45*, 517 (1970).
15 J. Das, in: In Search of the Site of Action of Plant Growth Substances, Ph. D. thesis. Kalyani University (1974).

Interaction of platinum compounds with bacterial DNA[1]

M. Tamaro, S. Venturini, C. Eftimiadi and C. Monti-Bragadin

Institute of Microbiology, University of Trieste, via Alfonso Valerio 34, I-34127 Trieste (Italy), 10 August 1976

Summary. The cis and trans isomer of $PtCl_2(NH_3)_2$, cis-$Pt(cpa)_2Cl_2$ and 2 platinum pyrimidine blues have been used in a number of bacterial tests indicative of their interaction with bacterial DNA.

cis-Dichlorodiammineplatinum (II) cis-$PtCl_2(NH_3)_2$ has been shown to induce point mutations of the base pair substitution type in E. coli[2] and S. typhimurium[3], and has a number of effects on bacterial systems, such as induction of filamentous growth in E. coli B[4], induction of prophage[5] and stronger toxic activity for repair deficient bacterial strains[6,7]. cis-$PtCl_2(NH_3)_2$ is also an antitumor agent[8,9] which inhibits in vivo[10] and in vitro the synthesis of DNA in eukariotic cells[11] and interacts with DNA causing interstrand crosslinks[8,12,13].

Table 1. Bacterial strains used

Strain designation	Characteristics	Source and reference
E. coli WP2	trp⁻	Venitt[16]
E. coli WP2 recA	same as WP2 except recA⁻	Venitt[16]
E. coli WP2 lexA	same as WP2 except lexA⁻	Venitt[16]
E. coli WP2 uvrA	same as WP2 except uvrA⁻	Venitt[16]
E. coli WP2 lexA uvrA	same as WP2 except lexA⁻ and uvrA⁻	Venitt[16]
E. coli AB 1157	thr⁻ leu⁻ pro⁻ his⁻ thi⁻ arg⁻	Bachmann[17]
E. coli AB 2463	same as AB 1157 except recA⁻	Bachmann[17]
E. coli AB 1886	same as AB 1157 except uvrA⁻	Bachmann[17]
E. coli AB 2480	thi⁻ pro⁻ recA⁻ uvrA⁻	Bachmann[17]
E. coli B	prototrophic	ATCC
E. coli J 53	pro⁻ met⁻ λ⁺	Bachmann[17]
E. coli W3110 thy⁻	thy⁻ λ⁻	De Lucia and Cairns[18]
S. typhimurium TA92	his⁻/pKM101	McCann et al.[19]
S. typhimurium TA100	same as TA92 except uvrB and rfa	McCann et al.[19]

It is well known that the trans-isomer acts in bacteria in a different way[7] and has no antitumor activity. The purpose of this report is to test the activity on several bacterial systems of cis and trans isomer of $PtCl_2(NH_3)_2$, of 2 platinum pyrimidine blues, Pt-uracil and Pt-thymine, which, according to Davidson et al.[14], contain 2 ammonia ligands, 1 pyrimidine anion and 1 hydroxide ion per platinum with 2 additional oxygen atoms at an unspecifiable location and of cis-[$Pt(cyclopentylamine)_2Cl_2$], cis-[$Pt(cpa)_2Cl_2$], an alyciclic primary amine of Pt (II). cis-[$Pt(cpa)_2Cl_2$] and platinum pyrimidine blues are interesting antitumor agents which have a spectrum of activity against tumors somewhat different from that of cis-$PtCl_2(NH_3)_2$[15], but show a higher therapeutic index than

1 This investigation was supported by a grant from C.N.R., Rome. We thank Mrs S. Saincich for skilful technical assistance.
2 D. J. Beck and R. R. Brubaker, Mut. Res. *27*, 181 (1974).
3 C. Monti-Bragadin, M. Tamaro and E. Banfi, Chem. Biol. Int. *11*, 469 (1975).
4 E. Renshow and A. J. Thomson, J. Bacteriol. *94*, 1915 (1967).
5 S. Reslova, Chem. Biol. Int. *4*, 66 (1971).
6 D. J. Beck and R. R. Brubaker, J. Bacteriol. *116*, 1247 (1973).
7 C. Monti-Bragadin, L. Ramani, L. Samer, G. Mestroni and G. Zassinovich, Antimicrob. ag. Chemother. *7*, 825 (1975).
8 B. Rosenberg, Naturwissenschaften *60*, 399 (1973).
9 B. Rosenberg, Cancer Chemother. Rep. *59*, 589 (1975).
10 J. A. Howle and G. R. Gale, Biochem. Pharmac. *19*, 2757 (1970).
11 H. C. Harder and B. Rosenberg, Int. J. Cancer *6*, 207 (1970).
12 G. R. Gale, in: Platinum compounds in antineoplastic and immunosuppressive agents, vol. 2, p. 829. Ed. A. C. Sartorelli and D. G. Johns. Academic Press, New York 1975.
13 L. L. Munchausen, Proc. Nat. Acad. Sci. USA *71*, 4519 (1974).
14 J. Davidson, P. Faber and R. Fischer. Cancer Chemother. Rep. *59*, 287 (1975).
15 P. D. Braddock, T. A. Connors, M. Jones, A. R. Khornar, D. H. Melzack and M. L. Tobe, Chem. Biol. Int. *11*, 145 (1975).