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].

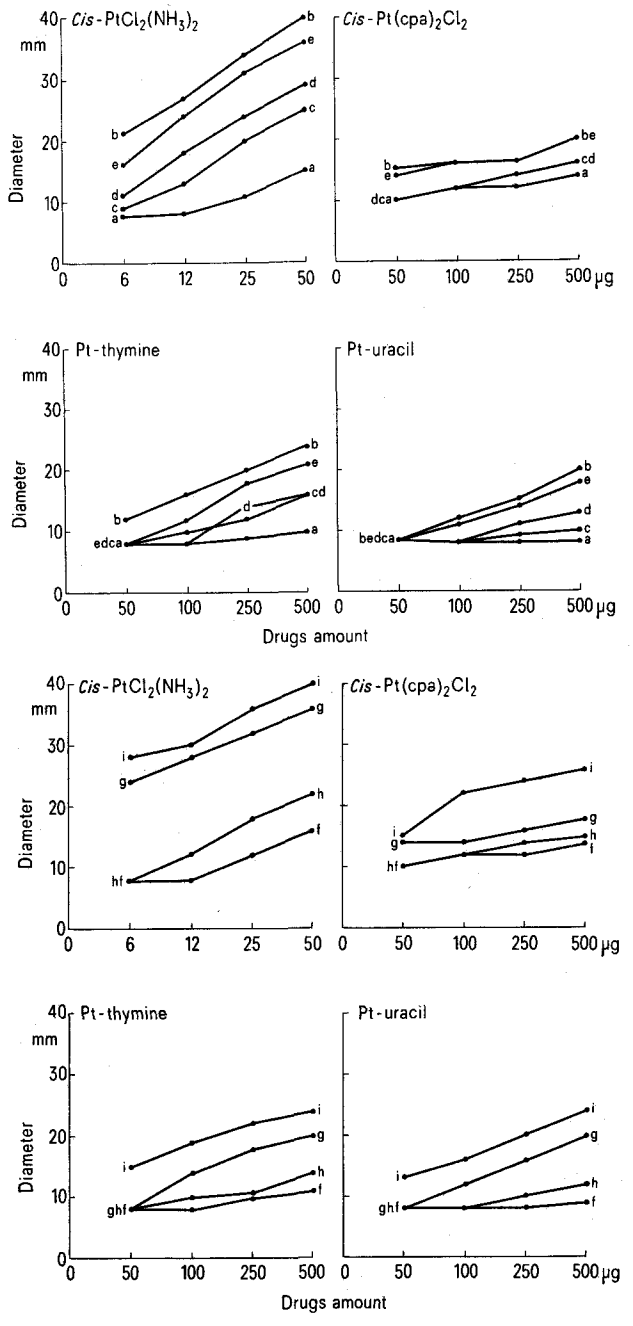
Table 1. Bacterial strains used

| 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).

the latter compound. The bacterial strains used are listed in table 1. cis- and trans-$PtCl_2(NH_3)_2$ were prepared as described[20] and were kindly supplied by G. Mestroni. cis-$[Pt(cpa)_2Cl_2]$ was generously donated by M. L. Tobe and the platinum pyrimidine blues: Pt-thymine and Pt-uracil by B. Rosenberg. The results of antibacterial activity of 4 platinum compounds on strains of E. coli




Antibacterial activity of various platinum (II) derivatives on E. coli WP2 (figure 1) and E. coli K12 (figure 2) strains with different repair capacities. For the antibacterial test 50 µl of appropriate dilutions of compounds were placed in 8 mm holes cut into pour plates with a lawn of bacteria. Davis-Mingioli synthetic medium[21] with adequate growth factors was used. The plates were then placed in a 37 °C incubator for 18 h and the zones of killing were measured.

*a* WP2, *b* WP2recA⁻, *c* WP2uvrA⁻, *d* WP2lexA⁻, *e* WP2lexA⁻uvrA⁻, *f* AB1157 (K12), *g* AB2463 (K12recA⁻), *h* AB1886 (K12uvrA⁻), *i* AB2480 (Kl2recA⁻uvrA⁻)

with different defects in DNA repair system, are reported in figures 1 and 2.

As expected the sensitivity to cis-$PtCl_2(NH_3)_2$ of single defective mutants are in the order recA⁻ > lexA⁻ > uvrA⁻[6,7]. The double defective strains AB 2480 and WP2 lexA⁻ uvrA⁻ are more sensitive than either single mutant. In contrast to this, no difference was found between the inhibition produced on the various strains by trans-$PtCl_2(NH_3)_2$ (data not shown). With minor differences, the sensitivities of the various strains to cis-$[Pt(cpa)_2Cl_2]$ and to pyrimidine platinum blues ranked in the same order as sensitivities to cis-$PtCl_2(NH_3)_2$.

Pt-thymine and Pt-uracil induced filamentous growth of E. coli at subtoxical concentrations as cis-$PtCl_2(NH_3)_2$. However, with the 2 pyrimidine blues, 100% of filamentous cells were not obtained even at the higher concentration tested. The lengths of the filament cells formed were from 10 times longer than normal cells. cis-$[Pt(cpa)_2Cl_2]$ was negative in filament formation as was the trans isomer of $PtCl_2(NH_3)_2$ (table 2).

When tested for lambda-inducing capacity by the plate test according to Mayer et al.[22], the 2 platinum pyrimidine blues were found to be positive along with cis-$PtCl_2(NH_3)_2$ while cis-$[Pt(cpa)_2Cl_2]$ and trans-$PtCl_2(NH_3)_2$ were consistently negative (table 2).

16 S. Venitt and L. S. Levy, Nature *250*, 493 (1974).
17 B. J. Bachmann, Bacteriol. Rev. *36*, 525 (1972).
18 P. De Lucia and Cairns, J. Nat. *224*, 1164 (1969).
19 J. McCann, N. E. Spingarn, J. Kobori and B. N. Ames, Proc. Nat. Acad. Sci. USA *72*, 979 (1975).
20 J. Kleinberg, in: Inorganic synthesis, vol. 7, p. 241. Ed. Mc Graw Hill, New York 1963.
21 B. D. Davis and E. S. Mingioli, J. Bacteriol. *60*, 17 (1950).
22 V. W. Mayer, M. G. Gabridge and E. J. Osvald, Appl. Microbiol. *18*, 697 (1969).

Table 2. Effect of platinum compounds in producing elongation in E. coli B and inducing lamda prophage

| Compounds | Filamentous cells (%)* concentration (µg/ml) 100 | 30 | 10 | 3 | 1 | Induction |
|---|---|---|---|---|---|---|
| cis-$PtCl_2(NH_3)_2$ | i | i | 100% | 20% | 0 | + |
| trans-$PtCl_2(NH_3)_2$ | i | i | i | 0 | 0 | – |
| cis$[Pt(cpa)_2Cl_2]$ | i | 0 | 0 | 0 | 0 | – |
| Pt-thymine | 50% | 50% | 30% | 10% | 0 | + |
| Pt-uracil | 50% | 50% | 30% | 10% | 0 | + |

*Abbreviations: i = inhibition of growth, 0 = no change in comparison controls.

Table 3. Number of induced his⁺ revertants for µmole of compound

| Compounds | S. typhimurium TA100 | Strain TA92 |
|---|---|---|
| Pt-thymine | 106 | 236 |
| Pt-uracil | 111 | 214 |
| cis-$[Pt(cpa)_2Cl_2]$ | 149 | 202 |
| cis-$Pt(NH_3)_2Cl_2$ | 7850 | 67500 |
| trans-$Pt(NH_3)_2Cl_2$ | 80 | 180 |

Using the mutagenic test as described by Ames[23], the platinum compounds were found to induce prototrophic revertants of S. typhimurium TA92 and TA100 strains, which carry his G 46, a mutation specifically reverted by basepair substitution mutagens[24] and pKM101, on R plasmid with mutator effect[19].

In table 3, a quantitative value of the mutagenic potential of the platinum compounds is given. On equimolar basis, cis-$PtCl_2(NH_3)_2$ induces 200–300 times more revertants in strain TA92, and 50–80 times more revertants in strain TA100, than the other compounds, which all show much the same activity.

The interaction with DNA has been claimed to be a prerequisite of most antitumor drug activity[25]. In this paper; 5 platinum compounds were used in 4 bacterial tests; filamentous growth[8], lambda induction[26], mutagenesis[23] and selective toxicity for DNA repair deficient strains, which are supposed to be indicative of interaction of a substance with bacterial DNA. With the antitumor activity of the tested compounds already known, the results can be used in the evaluation of the reliability of a given test identifying new antitumor drugs.

The results show that the tests for filamentous growth and for lambda induction fail to correlate with the interaction with bacterial DNA of one of the antitumor substances, cis-$[Pt(cpa)_2Cl_2]$, while the test for mutagenesis gave a weakly positive response even with trans-$PtCl_2(NH_3)_2$, which has no antitumor activity. The response of 3 out of the 4 antitumor platinum compounds is of the same order of magnitude, cis-$PtCl_2(NH_3)_2$ being the only substance which gives results clearly different from the ones of the inactive isomer. Only the test of selective toxicity for strains defective in DNA repair capacity was properly positive for 4 the antitumor compounds and negative for trans-$PtCl_2(NH_3)_2$.

23 B. N. Ames, F. D. Lee and W. E. Durston, Proc. Acad. Sci. USA *70*, 782 (1973).
24 H. J. Vogel and D. M. Bonner, J. biol. Chem. *218*, 97 (1956).
25 T. A. Connors, FEBS Letters *57*, 223 (1975).
26 B. Heinemann, in: Chemical mutagens. Ed. A. Hollander. Plenum Press, New York 1971.

## Some observations on the Rohon-Beard cell perikaryon

W. Meyer

*Institut für Zoologie, Tierärztliche Hochschule Hannover, Bischofsholer Damm 15, D-3000 Hannover (Federal Republic of Germany, BRD), 31 May 1976*

*Summary.* During the hatching period, the Rohon-Beard cells of Salmo gairdneri showed definite structural maturity of their perikarya, which is in contrast to the amphibian species investigated, where neuronal maturation of the Rohon-Beard cells is finished only in later – posthatching – developmental stages.

The Rohon-Beard cells (RBCells) are found as transitory neurons in the dorsal part of the developing spinal cord of most fish and amphibian species. These neuronal elements represent the sensory part of a primitive spinal reflex mechanism (primary reflex mechanism[1]). The RBCell axons form a dorsolaterally situated primary sensory tract in the spinal cord. Their branches leave the cord and reach the skin as free nerve endings; additional branches enter the dorsal parts of the myotomes[2, 3].

This paper presents a short account of the ultrastructure of the developing RBCell perikaryon with special regard to the hatching period of the fish and amphibian embryos and larvae investigated. The observations are discussed in relation to the possible biological significance of the RBCells.

*Materials and methods.* Embryos and larvae of the following fish and amphibian species were reared from eggs and their developmental stages were classified according to normal tables: Salmo gairdneri, stages 28–33[4]; Triturus helveticus, stages 33–38[5]; Xenopus laevis, stages 31–44[6]; Rana temporaria, stages 11–18[7]. The animals were fixed in 3.5% phosphate buffered glutaraldehyde, washed several times in a sucrose-phosphate buffer solution, and postfixed in 1% osmium tetroxide[8]. After dehydration in ethanol, the material was embedded in Araldit (Ciba) or Durcupan (Fluka)[9]. Thin sections were cut with a Reichert ultramicrotome (type Om U 2), stained with methanolic uranyl acetate and lead citrate[10, 11], and examined in the Siemens Elmiskop I A electron microscope.

*Results.* Within the developmental range covered by this investigation, neuronal maturation of the RBCells was manifested as typical structural changes of certain cell organelles. The most important ones are described in detail below:

In early stages before hatching (Salmo, stages 28–29; Triturus, stages 33–34; Xenopus, stages 31–33; Rana, stage 11) the endoplasmic reticulum (ER) mainly consisted of several shorter and dilated cisterns, not arranged in a parallel manner and without ribosomes on their surfaces, so that the smooth ER type dominated. Free ribosomes were uniformly distributed throughout the whole cytoplasm. During the subsequent developmental stages, the number of ribosome studded cisterns of the now rough ER increased, showing typical elongation and parallel arrangement (Nissl-substance) in the cell periphery. In an equal manner, the number of neurotubules, neurofilaments (figure 1) and mitochondria increased, the latter occasionally visible as very long, branched or

1 G. E. Coghill, Arch. Neurol. Psych., Chicago *21*, 989 (1929).
2 W. Meyer, Untersuchungen zur Struktur und Histochemie der Rohon-Beard-Zellen bei Fischen und Amphibien. Diss. T. U. Hannover 1974.
3 R. Nieuwenhuys, Prog. Brain Res. *11*, 1 (1964).
4 J. M. Vernier, Ann. Embryol. Morph. *2*, 495 (1969).
5 L. Gallien and O. Bidaud, Bull. Soc. zool. Fr. *84*, 22 (1959).
6 P. D. Nieuwkoop and J. Faber, Normal Table of Xenopus laevis (Daudin). North Holland Publ. Comp., Amsterdam 1967.
7 F. Kopsch, Die Entwicklung des Braunen Grasfrosches *Rana fusca* Roesel. Thieme, Stuttgart 1952.
8 G. Millonig, J. appl. Phys. *32*, 1637 (1961).
9 J. H. Luft, J. biophys. biochem. Cytol. *9*, 409 (1961).
10 E. S. Reynolds, J. cell. Biol. *17*, 208 (1963).
11 J. H. Venable and R. Coggeshall, J. cell. Biol. *25*, 407 (1965).