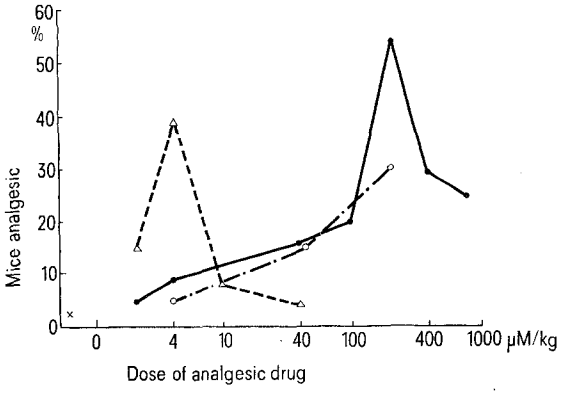

Fig. 1. Alteration of tail-clip analgesia in mice by 3-carboxysalsolinol, with or without carbidopa pretreatment, and by L-DOPA. At least 30 mice were used in each experiment. Analgesia testing was conducted 30 min after injections. The responses to 3cSAL (from 40 to 800 μM/kg) and to L-DOPA (40 and 200 μM/kg) are significantly different from the acidic saline control ($p < 0.05$). The response to 3cSAL (4 μM/kg) with CD pretreatment is significantly different from the non-pretreated control ($p < 0.015$). ×, Acidic saline control; ○–.–.– L-DOPA; ●——, 3-carboxysalsolinol; △– – –, 3-carboxysalsolinol with carbidopa (120 μM/kg) pretreatment.

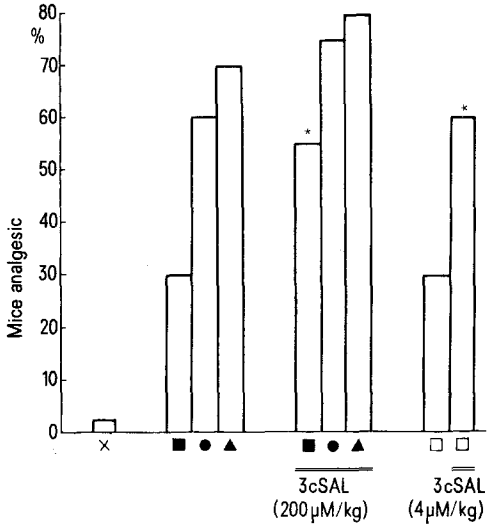

Fig. 2. Alteration of tail-clip analgesia in mice by morphine with 3-carboxysalsolinol co-treatment and carbidopa pretreatment. At least 30 mice were used in each experiment. The analgesia tests were conducted 30 min after the injections. An asterisk (*) indicates values significantly different from the 13 μM/kg morphine value ($p < 0.05$). ×, Acidic saline control; ■, morphine (13 μM/kg); ●, morphine (20 μM/kg); ▲, morphine (27 μM/kg); □, morphine (13 μM/kg) with carbidopa (120 μM/kg).

(200 μM/kg) produced enhanced levels of analgesia although significance was demonstrated with only the lowest M dose (13 μM/kg). CD did not alter analgesia associated with this dose of M. However, the combination of M (13 μM/kg) and 3cSAL (4 μM/kg) in CD-treated mice resulted in a significant increase in the level of analgesia. In contrast, L-DOPA (200 μM/kg) had no influence on analgesia produced by M. It was similarly unaltered in CD-treated mice receiving the low dose of M and L-DOPA (4 μM/kg).

Blumberg et al. first demonstrated the ability of N to counteract the actions of opiates[11]. In the present study, co-treatment with N abolished analgesia associated with the maximal responses to 3cSAL, 3cSAL with CD and L-DOPA (figure 1), and with M and combinations of M and other substances (figure 2).

The evidence presented here shows that 3cSAL, or its decarboxylated product SAL, can express an analgesic response that is antagonized by N. This suggests the possibility of an action on central opiate receptors[12]. Recent work has shown that SAL has affinity for the opiate receptors in the field-stimulated guinea-pig ileum[13] on which it acts as a weak agonist-antagonist. The biphasic analgesia pattern evinced by 3cSAL may represent a higher affinity of the isoquinoline for the agonist receptomer with increasing interaction with the antagonist configuration occurring at greater concentrations.

Co-treatments of 3cSAL (200 μM/kg) and M consistently increased the level of analgesia over that of M alone. The application of the conservative evaluative sample proportions method of analysis revealed that the accepted level of significance was achieved with only the lowest dose of M. Even so, this cannot be considered to reflect an additive M-3cSAL interaction as the increased level of analgesia is not significantly different from that produced by the 3cSAL alone. The possibility of such an interaction occurring is, however, suggested by the study in which 3cSAL (4 μM/kg) was combined with the low dose of M in CD-pretreated mice. In this case, the elevation of analgesic responses were significantly higher than those of M alone, M in decarboxylase inhibited animals and of the isoquinoline in the pretreated mice. This study indicates that 3cSAL, or SAL, can produce opiate-associated analgesia. The present in vivo results provide some endorsement for hypotheses that isoquinolines may unify ethanol and opiate dependencies[14,15].

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Effects of carmine and carminic acid on embryonic tissue cell cultures

L. Marzona, O. M. Olivo, G. Volpi and G. Toni

Anatomical Institute, Medical Faculty University of Modena, via Berengario 16, I-41100 Modena (Italy), 5 November 1976

Summary. The biological reaction to carmine and carminic acid at cellular level on 'in vitro' cultures was tested and significant variables were controlled. Results suggested that proliferation and metabolism of these cultures were not affected by the 2 stains.

Recently[1,2] it was suggested that carmine induced damaging action with teratogenetic effects in mice. In the present work, the biological reactions at the cellular level on both hanging-drop and monolayered 'in vitro' tissue cultures were carefully studied. Cultures were from embryonic myocardium and tendons of chicken and several mammalian species. Carmine or carminic acid solutions were supplemented to the culture medium (plasma and

chicken embryonic extract) to obtain final concentrations of 25 γ/ml–1250 γ/ml. The significant variables controlled in the hanging-drop cultures were: the average velocity of migration, the contractile activity of the myocardium explants, the morphology of migrated cells and the mitotic growth index.

The autonomous contractility of the myocardium explants was not altered by carmine or carminic acid. With the carmine, the average radial increase of the growth area varied from –5% to+3% with respect to the controls. These differences were not significant because of the notable variability normally present in the controls. The mitotic index of the primary cultures was very variable. Their average values, in groups consisting of 4–16 cultures treated with carmine or carminic acid, were equal, superior or inferior to those of the controls. For this reason, it is possible to say that carmine or carminic acid do not depress the proliferative activity of the cells.

In cultures supplemented with 100 γ/ml carmine solution, no cytomorphological differences with respect to the controls were revealed by light microscopy. With 500 γ/ml–1000 γ/ml solutions, masses of stained granules were found only in a very limited number of cells. Probably these are histiocyte-like elements. Similar masses were also observed with 5000 γ/ml solutions in almost all of migrating elements, but in smaller amounts than those presented with the histiocytes. Carminic acid has not been demonstrated as a specific endocellular deposit.

The morphology of the greater part of various mitosis phases was normal with the 2 dyes. Electron microscopy showed no specific morphological alterations. Well-conserved myofibrills as well cellular organelles, were observed in the explants.

It can be concluded that, in the range of doses employed, no harmful effects on the metabolism and the proliferation of 'in vitro' tissue cell cultures were manifested by the 2 staining agents.

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Daunomycin-bands are similar to Q-bands on chromosomes of Vicia faba

D. Gill and G. Nadjar[1]

Departments of Physics and Biology, Ben Gurion University of the Negev, Beer Sheva (Israel), 7 October 1976

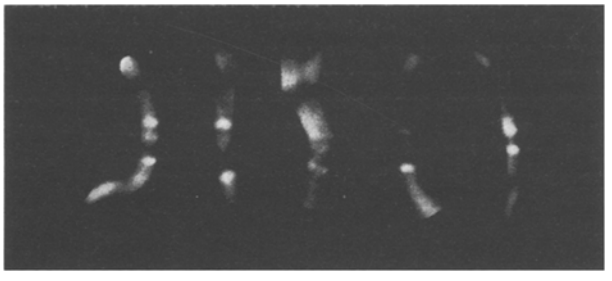
Summary. Lin et al.[2] discovered fluorescent bands on human chromosomes stained with daunomycin (D-bands). These bands looked like Q-bands. We demonstrate D-bands, which look like respective Q-bands, in Vicia faba and infer that the similarity between D and Q banding is general.

The anti-tumour drug daunomycin has an anthracycline chromophore constituent, which binds to DNA by intercalation[3–5]. Recently, the drug was applied to fixed metaphase chromosomes and its fluorescence revealed banding patterns[2]. In human metaphase chromosomes, D-bands were similar to Q-bands and in interphase nuclei the Y-bodies could be clearly discerned.

This finding was important because anthracycline represented a new family of chromosome-banding dyes. Also the rate of fading of daunomycin fluorescence was slower than of quinacrine dihydrochloride, thus allowing more leisurely approach to the samples. The near identity of D and Q bands in human chromosomes prompted us to make the comparison on the broad bean, Vicia faba, the Q-bands of which were reported in great detail[6–10].

Side roots of V. faba (var. major) seedlings were treated in 0.2% colchicine for 6 h and fixed overnigt in cold 3:1 ethanol-acetic acid. Next day the roots transferred to abs. ethanol and refrigerated until use. Before use, 1 or 2 root tips were brought to water, macerated for 1 min in 0.2 N HCl at 60 °C and rinsed 3 times in distilled water. The duration of the HCl treatment was critical, hence we chose to treat no more than 2 root tips at one time.

A root tip was put on a microscope slide, cut down to 3 mm length and covered with a drop of staining solution. Squashing was done in 2 steps, so as to guarantee good penetration of the stain. The covered slide was ready for observation, as there was no need for removal of excess stain. The staining solution was 0.5% daunomycin (Serva No. 18115) in demineralized water, the pH of which was 6 before the dye was dissolved. Staining at pH 4.3, recommended by Lin et al.[2] for human chromosomes, was ruinous for Vicia chromosome bands. A fluorescence microscope with incident illuminaction (Leitz Ortholux II)



Daunomycin (D-)bands on M-chromosomes arranged from C-metaphase plates of Vicia faba. The D-bands are similar to the respective Q-bands.

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