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.

1 G. Schlüter, Naunyn-Schmiedebergs Arch. Pharmak. *270*, 56 (1971).
2 G. Schlüter, Naunyn-Schmiedebergs Arch. Pharmak. *270*, 316 (1971).

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

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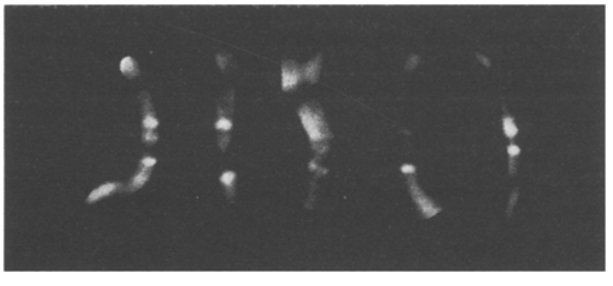
Summary. Lin et al.[2] discovered fluorescent bands on human chromosomes stained with daunomycin (D-bands). These bands looked like Q-bands. We demonstrate D-bands, which look like respective Q-bands, in Vicia faba and infer that the similarity between D and Q banding is general.

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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2 C. C. Lin and J. H. Van de Sande, Science *190*, 61 (1975).
3 E. Calendi, A. DiMarco, M. Reggiani, B. Scarpinato and L. Valentini, Biochim. biophys. Acta *103*, 25 (1965).
4 W. J. Pigram, W. Fuller and L. D. Hamilton, Nature New Biol. *235*, 17 (1972).
5 H. Kersten and W. Kersten, Molec. Biol. Biochem. Biophys. Ser. *18* (1974).
6 T. Caspersson, S. Farber, G. E. Foley, J. Kudynowsky, E. J. Modest, E. Simonsson, U. Wagh and L. Zech, Expl Cell Res. *49*, 219 (1968).
7 T. Caspersson, L. Zech, E. J. Modest, G. E. Foley, U. Wagh and E. Simonsson, Expl Cell Res. *58*, 128 (1969).
8 T. Caspersson, L. Zech, E. J. Modest, G. E. Foley, U. Wagh and E. Simonsson, Expl Cell Res. *58*, 141 (1969).
9 C. G. Vosa, Chromosoma *30*, 366 (1970).
10 C. E. Vosa and P. Marchi, G. Bot. ital. *106*, 151 (1972).

was used. Fluorescence was excited by the green line (546 nm) of a 200 W Hg lamp and the barrier filter passed wavelengths longer than 580 nm. Exposure time for the negative (100 ASA film) was 120 sec.
Chromocenters in interphase nuclei glowed brilliantly. In the metaphase M-chromosomes, the D-bands showed with marked contrast (figure) and were similar to the respective Q-bands[6–10]. The most intense band was on the long arm, close to the centromere, and 2 major and one minor bands were on the opposite side of the centromere, on the arm having the secondary constriction. This observation, combined with that on human chromosomes, lends support to the notion that D-bands are similar to Q-bands in all cases.

Enzymatic patterns in reptilian brain. Histochemical characterization of the optic tectum

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Summary. The enzymatic patterns present in the optic tectum of 4 species belonging to different reptilian orders seem related to the degree of structural and functional organization reached by the nervous centre, as in other vertebrates. In particular the AChE localization in reptiles is representative of a evolutionary sequence in the distribution of this enzyme in the optic tectum along the tetrapode series.

The optic tectum of non-mammalian vertebrates is usually a well-developed brain region which in several classes reaches high levels of structural and functional organization[1–3]. Following up a series of research on histoenzymological characterization of nervous centres in lower vertebrates[4–8], we have studied the histochemical localization of 5 enzymatic activities in the optic tectum of 4 species representative of the main reptilian orders. To our knowledge the histochemical studies on reptilian optic tectum have up till now only used turtle species[9,10].
Materials and methods. We have studied in the optic tectum of lizards (Lacerta muralis), snakes (Natrix natrix), turtles (Pseudemys scripta) and alligators (Caiman sclerops) the following enzymatic activities: acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), monoamine oxidase (MAO), lactate dehydrogenase (LDH) and succinate dehydrogenase (SDH).
For AChE and BuChE demonstration, the brains were fixed for 3–4 h in 10% formol saline at 4 °C, rinsed for 20 min in 0.1 M acetate buffer (pH 6), frozen and cut in the cryostat in transverse or sagittal planes. The sections, 30 µm in thickness, were incubated for 90 min at 22–24 °C in the media of Gerebtzoff[11] or Karnovsky and Roots[12] containing alternatively acetylthiocholine or butyrylthiocholine iodide as substrate. In order to test the actual nature of the enzymatic activity revealed, the following inhibitors were routinely used in a pre-incubation bath (45 min in acetate buffer containing the inhibitor) and in the final medium: the selective AChE inhibitor BW 284C51 5×10^{-5} M, the selective pseudocholinesterase inhibitor iso-OMPA 3×10^{-5} M and the inhibitor of all cholinesterases eserine 3×10^{-5} M[13–16]. For MAO, LDH and SDH demonstration, the unifxed brains were immediately frozen and cut in the cryostat at 20 µm thickness. The sections, adherent to coverslips, were briefly dried in the air and incubated for 45 min at 35 °C in the media of Glenner et al.[17] for MAO or the standard media for LDH and SDH[17]. As control of MAO reaction, some sections were treated in the pre-incubation bath (20 min at 35 °C) and in the final medium with the MAO inhibitor nialamide at 5×10^{-5} or 1×10^{-4} M concentration.
Results. AChE activity is usually weak in deeper tectal layers, and weak to moderate in the stratum griseum centrale while it is always present in the stratum fibrosum and griseum superficiale of Huber and Crosby[18], showing noticeable differences in distribution patterns among the different reptilian species examined. In Natrix (figure 1) the histochemical reaction is weak and uniformly spread all over the layer. In Lacerta (figure 2) and Pseudemys a similar distribution pattern exists: the middle band of the stratum fibrosum and griseum superficiale, largely corresponding to the stratum fusiforme retinum of Leghissa[2], shows a very strong reaction while the 2 adjacent plexiform layers exhibit much lower AChE activity. In Caiman (figure 3), the stratum fibrosum and griseum superficiale shows a clear laminar pattern with 2 bands of strong AChE activity severed by a wider band with moderate reaction. In the stratum opticum, the histochemical reaction is moderate or negative; in Lacerta and Pseudemys the stratum opticum is overlaid by a thin stratum zonale[1] showing moderate reaction. BuChE activity is not appreciable in the optic tectum of Pseudemys and Caiman; a very weak reaction for BuChE is present in Natrix and a more intense one in Lacerta (figure 4), both localized at level of fibrous tectal layers. The histochemical controls have confirmed the respective localization of AChE and BuChE: the reaction recorded with media containing acetylthiocholine is not sensitive to iso-OMPA while is inhibited by BW 284C51; the contrary

1 C. U. Ariens Kappers, J. C. Huber and E. C. Crosby, The Comparative Anatomy of the Nervous System of Vertebrates including Man, vol. 2. Hafner, New York 1960.
2 S. Leghissa, Arch. ital. Anat. Embriol. *68*, 343 (1962).
3 G. Szekely, in: Handbook of sensory Physiology, vol. VII/3B, p. 1. Ed. R. Jung. Springer Verlag, Berlin 1973.
4 A. Contestabile, Histochemistry *44*, 123 (1975).
5 A. Contestabile, Brain Res. *99*, 425 (1975).
6 A. Contestabile and I. Zannoni, Histochemistry *45*, 279 (1975).
7 A. Contestabile and I. Mussoni, Experientia *32*, 94 (1976).
8 A. Contestabile, Experientia *32*, 1223 (1976).
9 T. Kusunoki, J. Hirnforsch. *11*, 477 (1970).
10 G. Minelli, Riv. Biol. *63*, 61 (1970).
11 M. A. Gerebtzoff, Acta anat. *19*, 366 (1953).
12 M. J. Karnovsky and L. Roots, J. Histochem. Cytochem. *12*, 219 (1964).
13 B. J. Bayliss and A. Todrick, Biochem. J. *62*, 62 (1956).
14 L. Eranko, Histochemistry *33*, 1 (1973).
15 M. G. Ord and R. H. S. Thompson, Biochem. J. *51*, 245 (1952).
16 A. G. E. Pearse, Histochemistry. Theoretical and Applied, vol. 2. Churchill Livingstone, London 1972.
17 G. G. Glenner, H. J. Burtner and G. W. Brown, J. Histochem. Cytochem. *5*, 591 (1957).
18 G. C. Huber and E. C. Crosby, J. comp. Neurol. *57*, 57 (1933).