of avoidance responding was observed. In the group treated with bicuculline, escape responses were always at a 100% level. Furthermore, our data show that bicuculline, at a dose counteracting the effects of diazepam on rats in a conflict situation (1 mg/kg)[2], does not antagonize the disruption of avoidance performance induced by chlordiazepoxide (20 mg/kg) in previously trained rats (figure). In this last group, escape responses were at a 52.50% level. Conversely, it has been shown that chlordiazepoxide counteracts the disruptive effects of strychnine, a glycine antagonist, on avoidance behavior in mice[11].

Whether a GABAergic system plays a role in determining the facilitating effects of chlordiazepoxide on avoidance behavior in rats is not yet known, and should be investigated.

1 L. Stein, C.D. Wise, J.D. Belluzzi, in: Mechanism of action of benzodiazepines, p.29. Ed. E. Costa and P. Greengard. Raven Press, New York 1975.
2 V.V. Zakusov, R.U. Ostrovskaya, S.N. Kozhechkin, V.V. Markovich, G.M. Molodavkin and T.A. Voronina, Arch. int. Pharmacodyn. 229, 313 (1977).
3 P. Soubrie and P. Simon, Neuropharmacology 17, 121 (1978).
4 C.R. Gardner, T.G. Johns and P. James, 7th int. Congr. Pharmac. 1978, abstracts, p.498.
5 P. Soubrie, P. Simon and J.R. Boissier, Neuropharmacology 15, 773 (1976).
6 L. Cook and J. Sepinwall, in: Mechanism of action of benzodiazepines. Ed. E. Costa and P. Greengard. Raven Press, New York 1975.
7 A. Oliverio and C. Castellano, Psychopharmacologia 39, 13 (1974).
8 M. Sansone, Psychopharmacology 59, 157 (1978).
9 D. Bovet, G. Bignami and F. Robustelli, C. r. Acad. Sci., Paris, 266, 778 (1963).
10 V. Cuomo and A. Marino, Pharm. Res. Commun. 6, 531 (1974).
11 M. Sansone, Arch. int. Pharmacodyn. 215, 190 (1975).
12 P. Dagnelie, in: Théorie et méthodes statistiques, vol.2, p.252. Ed. J. Duculot. Gembloux, 1970.
13 A.J. Christmas and D.R. Maxwell, Neuropharmacology 9, 17 (1970).
14 A.S. Marriott and P.J. Spencer, Br. J. Pharmac. Chemother. 25, 432 (1965).
15 L.O. Randall and W. Schallek, in: Psychopharmacology, a review of progress 1957–1967. Publ. Hlth Serv. Publ. 1863, 153 (1968).
16 E.L. Abel, in: Drugs and Behavior, p.182. J. Wiley & So., New York 1974.
17 L. Cook and J. Sepinwall, in: Emotions, their parameters and measurement, p.379. Ed. L. Levi. Raven Press, New York 1975.
18 C. Morpurgo, Psychopharmacologia 8, 91 (1965).

Oxytalan fibres in human dental pulp[1]

Ž. Bradamante, A. Pećina-Hrnčević and I. Ciglar

Department of Histology and Embryology, Faculty of Medicine, University of Zagreb, Šalata 3, P.O. Box 166, YU–41001 Zagreb (Yugoslavia), and Department for Pediatric Dentistry and Department of Dental Pathology, Faculty of Dentistry, University of Zagreb, Gunduličeva 5, YU–41001 Zagreb (Yugoslavia), 19 November 1979

Summary. Scarce and randomly oriented oxytalan fibres are present in the connective tissue of dental pulp in both deciduous and permanent teeth.

Oxytalan fibres are histochemically defined on the basis of their stainability with acid orcein, aldehyde fuchsin and resorcin fuchsin only after previous oxidation. The stainability is abolished by digestion with testicular hyaluronidase after oxidation, but not by digestion with elastase prior to oxidation. On the ultrastructural level, the oxytalan fibres appear as bundles of 150-Å-thick microfibrils[2,3]. Histochemically and ultrastructurally identical fibres represent the first step in the formation of mature elastic fibres ('pre-elastic fibres')[3,4]. The oxytalan fibre, elaunin and mature elastic fibres are therefore referred to as the elastic system fibres[5].

The oxytalan fibres, as a definite connective tissue component, are predominantly located in the periodontal membrane of man and some mammals, but they were also found in some other anatomical sites. Fullmer[6] found oxytalan-like fibres in the pulp of the developing deciduous tooth. However, a description of these fibres in the human dental pulp is lacking even in the comprehensive reviews[7]. In the present communication, we show that oxytalan fibres can be regarded as a regular tissue component of the human dental pulp.

Material and methods. Pulps from 5 deciduous (6–9 years) and 6 permanent (12–58 years) teeth were examined. Permanent teeth were extracted for orthodontic reasons or because of periodontal disease. The dental cavity was widely opened from both sides and teeth were fixed in toto in Lillie's buffered formol. After 3–5 days in fixative, the pulp was excochleated, dehydrated, embedded in parafin

wax and sectioned serially at 7 μm. Deparafinized sections were examined after the following treatments[2,4]: Gomori's aldehyde fuchsin alone and after previous oxidation with peracetic acid, peracetic acid – aldehyde fuchsin – Halmi stain, PAS, Masson's trichrome stain, elastase – peracetic acid – aldehyde fuchsin, peracetic acid – testicular hyaluronidase – aldehyde fuchsin.

Results and discussion. No fibrillar structures in the pulp were stained with aldehyde fuchsin alone. The only exceptions were scarce elastic fibres around larger blood vessels. However, in sections stained with peracetic acid – aldehyde fuchsin without or with Halmi's counterstain, deep-purple, sharply outlined fibres were demonstrated in the intercellular matrix of the dental pulp of both deciduous and permanent teeth (figures 1 and 2). The interfibrillar matrix, as well as collagen fibres and nerves, stained very pale. In the central part of the pulp, the fibres were not oriented in any predominant direction but showed an apparent convergence to the walls of blood vessels. They were scarce in the central parts of the pulp and more numerous against its periphery. In some of the permanent tooth pulps, some wavy fibrils passed between odontoblasts. Fibres were not affected by pretreatment with elastase. However, they were not demonstrated in sections treated with testicular hyaluronidase between oxidation and staining with aldehyde fuchsin. The PAS staining also failed to demonstrate these fibres and only bundles of collagen fibres were demonstrated by the Masson's trichrome stain.

The results reported of histological staining show that fine
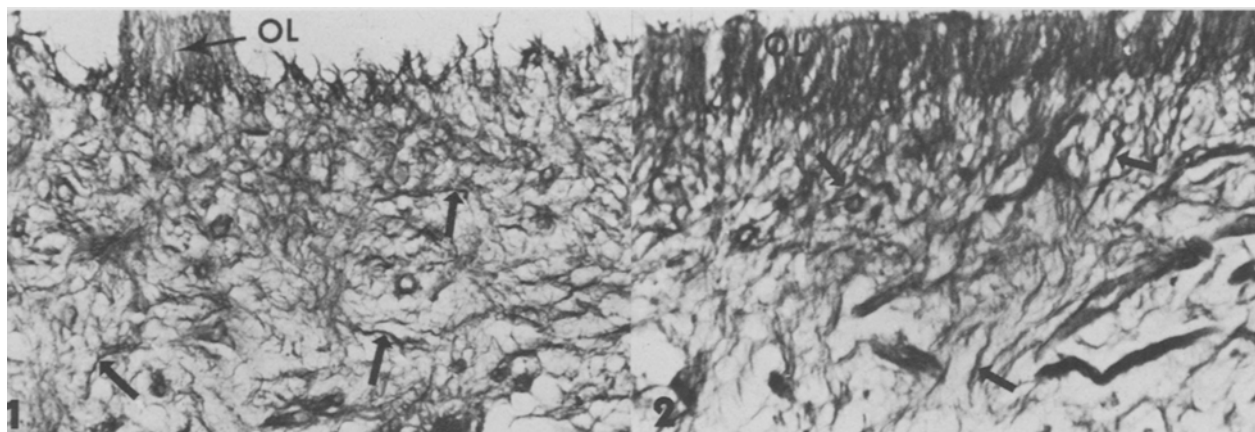
Figures 1 and 2. Human dental pulp. Fig. 1. 12 years old. Fig. 2. 43 years old. – Arrows point to oxytalan fibres in the central part of the pulp. Note penetration of fibres into the odontoblastic layer (OL). Peracetic acid-aldehyde fuchsin. × 270.

fibres in the dental pulp fulfil the light microscopical criteria for oxytalan fibres[2,3]. One may therefore conclude that oxytalan fibres are regular constituents of the intercellular matrix of the human dental pulp in both deciduous and permanent teeth. They very probably represent the light microscopical equivalent of the electron microscopical finding of unstriated 150-Å filaments similar to other microfilaments observed in the supporting tissues of the teeth[8].

The research on oxytalan fibres has been concerned mainly with their distribution and possible functional significance in the periodontal ligament. According to prevailing opinion, their function may be predominantly a mechanical one[2,9]. However, the presence of randomly oriented oxytalan fibres in the mechanically passive connective tissue of the dental pulp can hardly be explained in view of their mechanical role. The presence of oxytalan fibres also in the primitive mucous tissue of the umbilical cord[6], and the clear similarity between oxytalan fibres and invertebrate 'elastic fibres'[10], allow us to suppose that the presence of oxytalan fibres may be related to the primitive nature of the connective tissue in the dental pulp.

1 This investigation was supported by grant No. V-10/78 from the SIZ-V of S. R. Croatia.
2 H. M. Fullmer, J. H. Sheetz and A. J. Narkates, J. oral Path. 3, 291 (1974).
3 J. H. Sheetz, H. M. Fullmer and A. J. Narkates, J. oral Path. 2, 254 (1973).
4 Ž. Bradamante and A. Švajger, J. Anat. 123, 735 (1977).
5 G. Cotta-Pereira, F. Guerra Rodrigo and J. F. David-Ferreira, in: Elastin and Elastic Tissue, p. 19. Ed. L. F. Sandberg, W. R. Gray and C. Franzblau. Plenum Press, New York and London 1977.
6 H. M. Fullmer, Stain Technol. 34, 81 (1959).
7 C. C. Capuccino and R. F. Sheehan, in: Textbook of Oral Biology, p. 226. Ed. J. H. Shaw, E. A. Sweeney, C. C. Capuccino and S. M. Meller. W. B. Saunders Co., Philadelphia, London, Toronto 1978.
8 R. M. Frank and R. F. Sognaes, in: Histology, 4th edn, p. 615. Ed. L. Weiss and R. O. Greep. Mc Graw-Hill Book Co., New York 1977.
9 M. R. Sims, Am. J. Orthod. 70, 38 (1976).
10 H. Y. Elder, Biol. Bull. 144, 43 (1973).

Tumorigenic effect of topical mechlorethamine, BCNU and CCNU in mice

H. S. Zackheim[1] and E. A. Smuckler[2]

Departments of Dermatology and Pathology, University of California Medical Center, San Francisco (California 94143, USA), 27 December 1979

Summary. Mice were painted with mechlorethamine (HN2), carmustine (BCNU) and lomustine (CCNU) for up to 33 weeks. HN2 was a potent carcinogen, producing squamous cell carcinomas in 9 of 33 mice in 1 series. BCNU was a weak carcinogen. CCNU produced no tumors in 1 series.

This study reports the tumorigenic effect of topically applied mechlorethamine (nitrogen mustard, HN2); 1,3-bis-(2-chloroethyl)-1-nitrosourea (carmustine, BCNU); and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (lomustine, CCNU) in mice.
Although HN2 is carcinogenic when injected s.c.[3] or i.v.[4], in the only report of its topical application[5] painting with HN2 followed by croton oil resulted in warty growths but no carcinomas. BCNU and CCNU are intermediate strength carcinogens when injected in mice and rats[6].

Material and methods. All solutions were made fresh weekly and kept at 4–5 °C. Applications were made to the shaved mid-back of outbred female Swiss mice 8–10 weeks old. Only papillomas at least 1 mm in diameter were counted. All tumors were examined histologically.
Results. Results of series 1, 3 and 4 are summarized in the tables. In series 2 30 mice were painted with 0.1 mg of HN2 or BCNU in 0.2 ml 95% ethanol or with 0.2 ml 95% ethanol alone once weekly for 33 weeks. No tumors occurred in any group. Only a few of the HN2 and none of the BCNU mice