1 Westley, J.W., in: Polyether Antibiotics, Notation and Classification, vol. 1, p. 14. Marcel Dekker, New York and Basel 1982.
2 Westley, J.W., and Liu, C.M., US Patent, 4100171 (1978).
3 Liu, C.M., Hermann, T.E., Liu, M., Bull, D.N., Palleroni, N.J., Prosser, B., Westley, J.W., and Miller, P.A., J. Antibiotics *32* (1979) 95.
4 Bolte, J., Demuynck, C., Jeminet, G., Juillard, J., and Tissier, C., Can. J. Chem. *60* (1982) 981.
5 Duesler, E.N., and Paul, I.C., in: Polyether Antibiotics, vol. 2, p. 105. Marcel Dekker, New York and Basel 1983.
6 Duesler, E.N., and Paul, I.C., in: Polyether Antibiotics, vol. 2, p. 140. Marcel Dekker, New York and Basel 1983.
7 Westley, J.W., Evans, R.H., Sello, L.H., Troupe, N., Liu, C.M., and Blount, J.F., J. Antibiotics *32* (1979) 100.
8 Reed, P.W., Ionophores, in: Methods in Enzymology, vol. LV, p. 435. Academic Press 1979.
9 Dordick, R.S., Brierly, G.P., and Garlid, K.D., J. biol. Chem. *255* (1980) 1029.
10 Pyant, K.S., and Brierly, G.P., Experientia *38* (1982) 1202.
11 Gavach, C., Amblard, G., Sandeaux, R., Sandeaux, J., and Davion-Vanmau, N., in: Physical Chemistry of ion motions in membranes, Paris, 27 Sept–2 Oct. Ed G. Spach. Elsevier 1983.

0014-4754/84/060551-03$1.50 + 0.20/0

Rabbit chondrocytes are binucleate in auricular but not articular cartilage[1]

J.M. Lipman, B.J. Hicks and L. Sokoloff

Department of Pathology, State University of New York at Stony Brook, Stony Brook (New York 11794, USA), 21 June 1983

Ear cartilage chondrocytes have a single nucleus in the newborn rabbit but many become binucleate after 1 month of age[2–5]. Approximately 10% of articular chondrocytes in monolayer culture contain 2 nuclei[6]. It is not known whether this is true of the same cells in vivo. It has been reported that the mean DNA content of chondrocytes freshly isolated from the joints of rabbits is the same as that in culture[7]. If true, $^1/_{10}$ of the native cells may have more than 1 nucleus. The following study was undertaken to ascertain this point because aging of articular chondrocytes has often been proposed as the cellular basis of degenerative joint disease.

Materials and methods. Auricular and articular chondrocytes were isolated from 4 New Zealand White rabbits 0.3–4 years old (table 1). Absence of contaminating cell types following initial dissection was confirmed by histological examination of the cartilage. Chondrocytes were dissociated from the matrix by sequential digestion with hyaluronidase, trypsin and collagenase as described previously[8,9]. Because conventional smears of ear cells proved unsatisfactory, the chondrocytes were examined in histological sections. Articular chondrocytes were centrifuged into pellets directly and fixed in neutral buffered formalin. The procedure for auricular chondrocytes was modified slightly because their high fat content made them buoyant in the centrifuge. The cell suspensions were therefore first spun in Gey's balanced salt solution at 600 × g. The top 2 ml containing the floating cells were removed. Following aspiration of the remainder of the supernatant, the floaters were added to the cell pellet and the fluid made to 50% ethanol (v:v). This technical modification did not account for differences between the 2 cell types: an additional preparation of articular chondrocytes, employing the same ethanol-saline centrifugation step, yielded identical results. The suspension was recentrifuged and the resulting pellets processed as for the articular chondrocytes. They were embedded in JB4 glycomethacrylate (Polysciences, Warrington, PA), cut at a thickness of 2 µm, and stained with Giemsa[10]. The number of nuclei was counted in 400 cells of each group at a magnification of 1000 diameters. The dimensions of representative cells were measured with an eyepiece micrometer.

Results. In 3 rabbits 4–18 months old, 13.5–24.2% of auricular chondrocytes had 2 nuclei (table 1). 73% of these cells contained fat vacuoles. Double nuclei were seen in non-vacuolated as well as vacuolated cells (fig. A, B). The values presented may underestimate the actual proportion of binucleate auricular chondrocytes because the vacuolated cells were very large. The diameter was up to 29.8 µm while the length of the nuclei averaged 6.8 µm. An individual nucleus might thus not appear in a random section 2 µm thick. It is conceivable that false double nuclear profiles in auricular chondrocytes at times arose from bilobation or curvature of single nuclei. This is unlikely to account for the bulk of the binucleation observed because 1) auricular chondrocytes are binucleate and not bilobate in cell smear preparations; 2) the nuclei appeared discrete in adjacent sections of the same cell; and 3) articular chondrocytes, prepared by the same technique, appeared mononucleate.

By contrast, articular chondrocytes were not vacuolated. They were overwhelmingly mononucleate even up to 48 months of

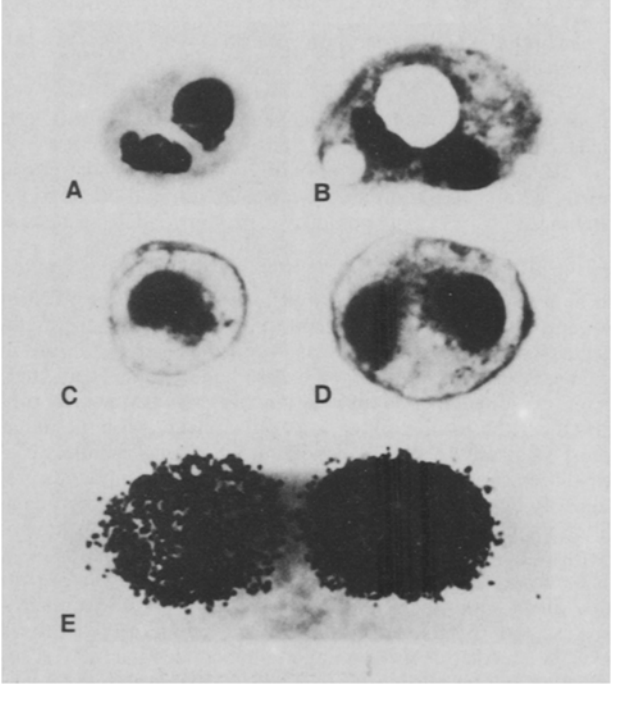

Figure 1. *A–D*, Sections of uncultured rabbit chondrocytes; *A*, binucleate ear chondrocyte that is not vacuolated; *B*, binucleate vacuolated ear chondrocyte; *C*, mononucleate articular chondrocyte; *D*, binucleate articular chondrocyte; *E*, Autoradiograph of ^{3}H-thymidine-labeled binucleate rabbit articular chondrocyte in monolayer culture. Giemsa, × 1500.

age (fig. C). Rare binucleate forms were seen (fig. D). In ^{3}H-thymidine-labeled cultured articular chondrocytes, both nuclei of binucleate cells had comparable numbers of grains (fig. E).

Discussion. The present results demonstrate a dichotomy between the replicative behavior of the 2 types of chondrocyte with respect to age in vivo. They provide no support for the occurrence of sizable numbers of binucleate chondrocytes to account for an apparently high DNA content in articular cartilage[7], but do not exclude polyploidy of mononuclear chondrocytes in the latter. Polyploidy and binucleation occur in mammalian tissues, particularly the liver. Polyploid and binucleate hepatocytes are capable of proliferating. Although there is a decline in the replicative activity of hepatocytes in aged animals, it is not the direct consequence of the polyploidy since the latter develops primarily in young individuals[11]. Auricular chondrocytes apparently do not divide in vivo but retain, despite their binucleation, a regenerative potential when cultured in vitro[4]. The mechanism for the binucleation of chondrocytes is not presently known. The apparent synchrony of DNA synthesis in the cultured chondrocytes illustrated in figure E does not in itself favor an acytokinetic mechanism over cell fusion. It has been demonstrated repeatedly for other cell types that fused homokaryons rapidly coordinate both DNA synthesis and mitosis[12]. Binucleation is a common finding in cultured articular chondrocytes[6, 13], as it is in many other animal cells in vitro. The phenomenon presumably is of stochastic origin, but its precise cause is unknown.

1 Supported by grant AM17258-08 from the National Institutes of Health, Robert Skinner and Susan Patterson made the sections. Lowell Kleinman participated in preliminary studies.
2 Moskalewski, S., and Rybicka, E., Acta anat. *97* (1977) 231.
3 Cox, R.W., and Peacock, M.A., J. Anat. *123* (1972) 283.
4 Moskalewski, S., Golaszewska, A., and Ksiazek, T., Experientia *36* (1980) 1294.
5 Adamiec, I.A., Acta histochem. *69* (1981) 264.
6 Krystal, G., Morris, G.M., Lipman, J.M., and Sokoloff, L., Mech. Ageing Devel. *21* (1983) 83.
7 Cheung, H.S., and Ryan, L.M., Analyt. Biochem. *116* (1981) 93.
8 Green, W.T. Jr, Clin. Orthop. *75* (1971) 248.
9 Sokoloff, L., Malemud, C.J., and Green, W.T. Jr, Arthritis Rheum. *13* (1970) 118.
10 Lillie, R.D., Histopathological Technique and Practical Histochemistry, 3rd edn. McGraw-Hill, New York 1965.
11 Brodsky, W.Ya., and Uryvaeva, I.V., Int. Rev. Cytol. *50* (1977) 275.
12 Johnson, R.T., and Rao, P.A., Biol. Rev. *46* (1971) 97.
13 Ronot, X., Hecquet, C., Jaffray, P., Guiguet, M., Adolphe, M., Fontagne, J., and Lechat, P., Cell Tissue Kinet. *16* (1983) 531.

0014-4754/84/060553-02$1.50 + 0.20/0

Model systems demonstrating the volatile mutagenicity and carcinogenicity of sodium nitrite in rats[1, 2]

I.A. Macdonald, J.A. Mader, B.G. Rao and D.A. Malatjalian

Departments of Medicine, Biochemistry and Pathology, Dalhousie University, Halifax (Nova Scotia, Canada B3H4H7), 3 September 1982

Summary. Volatile mutagens derived from sodium nitrite buffered at various pH values or in the presence of human feces were detected using Ames *Salmonella* tester strain TA 1535 on petrie plates inverted over samples. Volatile mutagenicity increased as the pH decreased and was primarily a function of the nitrous acid produced from sodium nitrite and hydrogen ions. Sodium nitrite administered intracecally to 3 Wistar rats through surgically implanted cannula caused tumors (fibrosarcoma: ⅓ and squamous cell, ⅔). The possible role of nitrite-derived mutagens in GI cancer is discussed.

The discovery of the presence of low amounts of sodium nitrite in human feces[3] has initiated our investigation of the effect of sodium nitrite deliberately added to human feces on the mutagenicity of the volatile products[4, 5] and the chemical nature of the gases involved[5]. Additionally, we have preliminarily characterized some ether-soluble, Griess test-positive material produced on the incubation of feces with nitrite[6]. Volatile mutagens obtained by steam distillation of normal human feces have been shown to be carcinogenic N-nitroso compounds presumably derived in vivo from nitrite[7, 8]. However, a non-volatile fat soluble mutagen recently purified from human feces is not a N-nitroso derivative[9, 10].

The purpose of the following communication is to demonstrate that direct acting non-microbially produced volatile mutagens are evolved from sodium nitrite in the presence of hydrogen ions. We further describe an animal model designed to test the effect of sodium nitrite (or other pharmacological, biochemical or microbial agents) on the large bowel.

Materials and methods. Collection of human fecal samples. Stool samples from healthy volunteers were collected in Zip-Loc bags.

Assay for volatile mutagens. We employed an Ames assay system[11, 12] modified for detection of volatile mutagens as described earlier[3]. Plates containing *Salmonella typhimurium* strain TA 1535 (a base pair substitution mutant) were prepared according to Ames[11, 12] without liver microsomes. Nitrite was added to stool samples at a final concentration of 0.6 M or 0.2 M. Similarly, nitrite was incubated without feces. Buffers were 0.2 M imidazole HCl (pH 6.0–7.2) and 0.2 M citrate (pH 4.4–6.0). Systems were incubated with exposure of plates to the volatile mutagens for 21 h at 37 °C and then the exposed plates incubated a further 27 h at 37 °C before counting the revertant colonies as described before[4].

Surgery. Young male Wistar rats weighing approximately 200–250 g were cannulized. The pre-sterilized cannula consisted of a hollow, flatheaded, stainless-steel bolt approximately 1.5 cm long, (o.d. = 2.0 mm, i.d. = 1.0 mm and 2 washers and 2 nuts; see fig. 1). A vertical incision approximately 3 cm long was made in the lower abdomen of the halothane-anesthetized animal and the cecum located. A purse-string stitch was performed and the head of the cannula plus the small stainless steel washer (approximately 8 mm in diameter) was inserted into the cecum, approximately 2.5 cm from the ileal cecal junction. The purse string was firmly closed. A second larger washer (teflon) 13 mm in diameter was placed on the cannula, which was passed through the abdominal wall via a small stab