rabbit red blood cells. Specificity was checked by inhibition with 0.3 M lactose.

Results. Hepatic lectin activity rose after partial hepatectomy and sham operation with manipulation of the liver by 12 h, peeked at 24–72 h, and declined by 144 h (table). Similar hemagglutination titers were obtained for both procedures, and in each instance values were inhibited by greater than 50% with 0.3 M lactose. The results suggest the lectin response correlates better with trauma than with cell proliferation since no difference in the lectin activity appeared between weak and strong regenerative stimulus. The function of the soluble hepatic lectin is not known. A possible explanation of our data lies in the recent identification of acute phase proteins with lectin properties[11,12], and the finding of soluble hepatic lectin in Kupffer cells[8]. The lectin may be related to the acute phase protein synthesized or released by the Kupffer cells in response to trauma.

Hepatic lectin in regenerating liver

Hours post surgery	Partial hepatectomy	Sham operated
6	72±10	57±16
12	477±157	641±95
24	550±50	492±103
48	422±82	-
72	442±51	600±138
144	23±4	47±17
192	53±15	69±51
Pre-hepatectomy liver 57±23		

Values are expressed as mean±SEM, hemagglutination titer^{-1}/mg protein.

1 G. Ashwell and A.G. Morell, Trends Biochem. Sci. *2*, 76 (1977).
2 T. Kawasaki and G. Ashwell, J. biol. Chem. *251*, 1296 (1976).
3 D.J. Bowles and H. Kauss, FEBS Lett. *66*, 16 (1976).
4 J.P. Prieels, S. Pizzo, L. Glasgow, J. Paulson and R. Hill, Proc. natl Acad. Sci. USA *75*, 2215 (1978).
5 T. Kawasaki, R. Etoh and I. Yamashina, Biochem. biophys. Res. Commun. *81*, 1018 (1978).
6 D.L. Simpson, D.R. Thorne and H.H. Loh, Nature *266*, 367 (1977).
7 T.P. Nowak, D. Kobiler, L.E. Roel and S.H. Barondes, J. biol. Chem. *252*, 6026 (1977).
8 E.C. Beyer, K.T. Tokuyasu and S. Barondes, J. Cell Biol. *82*, 565 (1979).
9 D. Kobiler, S.H. Barondes, Devl Biol. *60*, 326 (1977).
10 G.M. Higgins and R.M. Anderson, Archs Path. *12*, 186 (1931).
11 G. Uhlenbruck and D. Karduck, Naturwissenschaften *66*, 524 (1979).
12 G. Uhlenbruck, D. Karduck, H. Haupt and H.G. Schwick, Z. immun. Forsch. *155*, 262 (1972).

Response of maize to different inoculum densities of vesicular arbuscular mycorrhizal endophytes

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Summary. The effect of the inoculum density of vesicular arbuscular mycorrhizal endophytes on growth and development in maize was investigated in sterilized soil under glass-house conditions. Mycorrhizal plants grew robust and produced three times more dry weight than non-mycorrhizal plants. 40 or more endophytes per plant produced the highest mycorrhizal association and the maximum growth in maize. The uninoculated plants exhibited the symptoms of chlorosis.

Vesicular arbuscular mycorrhiza (VAM) may markedly influence the growth of the host plant[1] in a soil with low available phosphorus. Such increases are more noticeable in irradiated soil than unsterilized soil[2]. This may be ascribed to there being less microbial competition, and to other nutrient factors. In certain cases the plants lacking mycorrhiza may not survive[3]. The VAM has been widely exploited in the uptake of phosphorus from P-deficient soils[4–7]. To find a suitable inoculum, different levels of endophyte inoculum were tested for their effectiveness on growth and development in maize plants.

Methods. Sterilized maize seeds were germinated in sterilized moist chambers and these seedlings were transplanted (2-cm-long radicle stage) into pots (11 cm × 13 cm) filled with steam sterilized soil (pH 5.8, available phosphorus 0.03%, organic matter 3.2%, nitrogen 0.03% and potassium 2.4%). 5 maize plants were transplanted into each pot. The pots were subsequently inoculated with VAM endophytes (*Glomus* sp.) at different inoculum levels (2, 4, 16, 40 and 60 spores per plant). The uninoculated plants received doubly-sieved soil containing the soil microflora without any mycorrhizal propagules[8].

Growth of maize plants was measured (stem height, leaf number) on 20, 35 and 60 days after transplanting maize seedlings into pots. The shoot dry weight and mycorrhizal association were also recorded at these harvestings. Mycorrhiza was assessed by technique of Phillips and Hayman[9]. The percentage of infected roots was calculated using the roots which either possessed vesicles, arbuscule or both.

Results and discussion. Significant variation was observed in growth and development of mycorrhizal and nonmycorrhizal plants (table 1). At the 1st harvest (20 days) insignifi-

Table 1. Production of shoot dry weight, leaf number and stem height in relation to inoculum density

Spore number inoculated	Dry weight (g) I	II	III	F-value	Leaf number I	II	III	F-value	Stem height (cm) I	II	III	F-value
2	76.6	203.3	268.7	11.05**	3	6	11	37.47**	16.9	38.9	59.8	17.39**
4	70.0	193.3	265.8		4	7	12		21.8	36.4	57.8	
16	96.7	263.3	419.5		4	7	12		23.9	44.8	64.2	
40	104.3	356.6	578.4		4	8	13		25.8	53.03	74.4	
60	143.3	396.6	610.5		5	8	14		37.7	75.8	98.3	
Control	46.6	120.0	210.4		3	5	7		16.6	38.06	49.4	

I, 1st harvesting; II, 2nd harvesting; III, 3rd harvesting; F-value of analysis of variance; ** significant at 1% level.

cant variation in plant height between mycorrhizal and nonmycorrhizal plants was observed, but at later stages the variation was significant. The low inoculum produced less and late mycorrhizal association (table 2) whereas a high inoculum (40 and above endophytes per plant) developed fast and more VAM (table 1). A spore inoculum per plant of 40 or above produced about 3-fold more shoot dry weight than nonmycorrhizal plants. Non-inoculated plants exhibited the symptoms of chlorosis at later stages of harvestings (35 days).

A different pattern of growth and development in maize plants was obtained in response to different inoculum densities. An inoculum of 40 or more endophytes per plant showed a significant variation in growth of maize compared with nonmycorrhizal plants and mycorrhizal plants inoculated with a low level of endophyte. A positive correlation between inoculum density and growth of plants is reported[10]. In some studies[11] spore inoculation has been reported to delay the mycorrhizal association for various reasons, like the time required for spore germination, germ tube elongation and penetration into roots, but a higher inoculum may overcome such problems. The 3-fold increase in dry weight of mycorrhizal plants compared with nonmycorrhizal plants may be attributed to an increase in uptake of nutrients[1,12], by providing an additional absorbing surface for the root system of the host plant. Mycorrhiza not only absorbs phosphorus but also accumulates more phosphate and can solubilize the 'unavailable' phosphorus which is not available to nonmycorrhizal plants[13]. The present study suggests that mycorrhizal association is necessary for better growth. The inoculum level necessary for the enhanced growth, however, may vary in different plants and for various endophytes under different ecological conditions.

Table 2. Establishment of mycorrhiza in maize with respect to spore inoculation

Spore number inoculated	Mycorrhiza establishment (%)		
	1st harvesting (20 days)	2nd harvesting (35 days)	3rd harvesting (60 days)
2	-	6	8
4	-	7	12
16	-	15	22
40	8	30	68
60	12	36	70
Control	-	-	-

1 D.S. Hayman and B. Mosse, New Phytol. *70*, 19 (1971).
2 D.K. Paget, Endomycorrhizas. Ed. Sanders, Mosse and Tinker. Academic Press, New York 1975.
3 G.T.S. Baylis, N.Z.Jl Bot. *7*, 173 (1959).
4 C.Ll. Powell, New Phytol. *75*, 563 (1975).
5 J.W. Gerdemann, A. Rev. Phytopath. *6*, 397 (1968).
6 J.L. Harley, The Biology of Mycorrhiza. Leonhard Hill Publ. London 1969.
7 B. Mosse, A. Rev. Phytopath. *11*, 171 (1973).
8 C.Ll. Powell, New Phytol. *75*, 563 (1975).
9 J.M. Phillips and D.S. Hayman, Trans. Br. mycol. Soc. *55*, 158 (1970).
10 D.C. Carling, M.F. Brown and R.A. Brown, Can. J. Bot. *57*, 1769 (1979).
11 J.C. Sutton, Can. J. Bot. *51*, 2487 (1973).
12 A.G. Khan, New Phytol. *71*, 613 (1972).
13 S.E. Smith, CRC Critical Rev. Microbiol., June 1974, p.273.

Double minutes in fibroblast-like cells isolated from human tumors[1]

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Summary. Double minutes (DM) were found in several fibroblast-like lines isolated from human tumors. DM varied in size and morphology within the same metaphase.

Double minutes (DM) have been observed in metaphases of many tumor-derived epithelial cells[2-9] and never, to our knowledge, in normal cells[2,7]. In this report, we present our data on the incidence of DM in fibroblast-like cells originating from neoplastic and non-neoplastic tissues.

Materials and methods. Surgical specimens were obtained from patients who had not received chemotherapy or radiotherapy. Specimens from solid tumors were excised from the interior of the tumor in order to avoid possible contamination with surrounding normal tissue. Tissue pieces were trypsin-dissociated and transferred to culture flasks with McCoy's 5a medium supplemented with 15% fetal bovine serum, penicillin, streptomycin and amphotericin B. Most specimens gave growth of long spindle-shaped cells within 10–20 days. At this stage, supernatants were replaced with fresh medium and cells were allowed to form confluent monolayers; these were trypsinized and cell suspensions were transferred in new flasks. Epithelial cell growth was never noticed in flasks from which supernatant medium had been removed immediately after the first appearance of fibroblast growth. Cell morphology was examined on coverslip cultures stained with Giemsa. Chromosome preparations were made by conventional methods[10], after the 1st and up to the 10th passage of each finite cell line. Due to variable response of cell lines, colcemid doses which ranged from 0.06 to 0.3 μg/ml given for 3–8 h, were required in order to obtain reasonable preparations. All cells were free of contamination as shown by microscopic examination and by ^{3}H-thymidine labelling and autoradiography[11,12].

Results. 16 finite fibroblast-like cell lines were produced from tissue specimens obtained from patients with infiltrating ductal carcinoma (7 cases), chronic mastitis (4 cases), lymph node metastatic lesion of primary infiltrating ductal carcinoma (2 cases), nipple melanoma (1 case), trachelitis (1 case) and tonsillitis (1 case); samples of normal breast epidermis (2 cases) were also obtained from 2 patients with infiltrating ductal carcinoma. All lines used in this work consisted entirely of diploid or near-diploid fibroblast-like cells as confirmed by detailed microscopic examination. Several lines produced from malignant tissues exhibited a peculiar morphological pattern of growth: 'periodically appearing piling-up (PAPU) colonies' (fig. 1). Most of these colonies, observed in cell populations growing in culture flasks or on coverslips, resembled those observed by Giraldo et al.[13] in cultured sarcoma cells. These results will be presented in detail elsewhere.