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COMMUNICATIONS

Biocompatibility of wound management products: the effect of various monosaccharides on L929 and 2002 fibroblast cells in culture

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Abstract—The effects of various monosaccharides on the growth of human 2002 and mouse L929 fibroblast cultures have been investigated. Eleven monosaccharides having acidic, neutral, and basic characteristics were evaluated in a bioassay procedure developed for the investigation of biocompatibility of wound management materials. Rate of growth in both cell lines was inhibited by D-galacturonic acid and by D-glucuronic acid. Although most neutral sugars produced no significant change in the growth rate or in the morphology of the cells, galactose produced a significant increase in the growth rate of both cell lines whilst L-fucose caused a significant decrease in growth of the L929 cells but did not significantly affect the growth of 2002 cells; xylose increased the growth rate of L929 but not 2002 cells. D-Glucosamine, a basic sugar, produced inhibition of growth which followed a different pattern from that produced by the acidic sugars; *N*-acetylglucosamine produced a species specific increase in cell growth of L929 cells. The results show that the effects produced by the monosaccharides on the cultured fibroblasts are related to their chemical structure and to cell line, and suggest that the use of galactose as a possible aid to wound healing should be investigated.

Sugar (i.e. sucrose) and honey have been used to treat wounds for thousands of years and, according to Middleton & Seal (1985), granular sugar is in current use as an aid to wound healing in several centres throughout the world. Middleton & Seal (1985) formulated two sugar pastes based on caster sugar, additive-free icing sugar, polyethylene glycol (PEG) 400, and hydrogen peroxide and found these to be effective in the treatment of abscess cavities and sacral pressure sores. Over and above the evident in-vitro antimicrobial activity of PEG 400 and hydrogen peroxide, and the osmotic debriding properties of the pastes, a stimulating effect upon granulation was observed which could not readily be explained. Few if any studies have been made to elucidate the role of sugar(s) in/on the healing wound.

To investigate the likely value of simple sugars in wound healing, it is necessary to determine whether these compounds display any intrinsic biological activity in single cell lines from skin cultured in-vitro. Skin fibroblasts may be regarded as pertinent cell lines for such studies. The effects of various sugars as substitutes for glucose in fibroblast cultures have been recorded by Johnson & Schwartz (1976), Wolfrom et al (1983), Delhotal et al (1984), and others. The work reported here was undertaken to determine the effect of various sugars when present together with glucose in cultures of normal human fibroblasts and transformed L929 mouse fibroblasts using a bioassay method developed specifically for biocompatibility studies of wound management materials (Turner et al 1989).

Materials and methods

Cell Lines. L929 mouse fibroblasts and Flow 2002 human fibroblasts were obtained from Flow Laboratories, Rickmansworth, UK.

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Culture media. Unless otherwise stated, all materials were purchased from Flow Laboratories, Rickmansworth, UK.

Complete growth medium for L929 mouse fibroblasts consisted of Minimum Essential Medium Eagle (Modified) with Earle's salts (EMEM) containing 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid (HEPES) supplemented with non-essential amino-acids (1% dilution of MEM concentrate), 2 mM L-glutamine, and 10% foetal bovine serum (FBS).

Complete growth medium for 2002 human fibroblasts consisted of Basal Medium Eagle (Modified) with Earle's salts (EBME) containing 20 mM HEPES supplemented with non-essential amino-acids (1% dilution of BME concentrate), 2 mM L-glutamine, and 10% FBS.

Stock cultures were grown in 75 cm^2 flasks; 2·25 cm^2 multi(24)-well dishes were used in the assays. Antibiotics (penicillin 100 units mL^{-1} and streptomycin 100 μg mL^{-1}) were used in the stock cultures but not in the test dishes.

Dulbecco's phosphate buffered balanced salt solution (BSS) without Ca^{2+} or Mg^{2+} containing trypsin (0·25%) was used in cell trypsinization procedures.

Cell counting was carried out using an Improved Neubauer haemacytometer chamber (Astell Hearson) and the Coulter counter Model ZB as previously described (Turner et al 1989).

Sugars. D-Glucose, D-galactose, D-arabinose, D-xylose, D-mannose, L-rhamnose, L-fucose, D-galacturonic acid, D-glucuronic acid, 2-amino-2-deoxy-D-glucose (glucosamine), and 2-acetamido-2-deoxy-D-glucose (*N*-acetylglucosamine) were obtained from Sigma Chemical Company, Poole, Dorset, UK and used without further purification.

Routine maintenance of the cultures. Stock cells were stored in liquid nitrogen. New cultures were initiated from frozen stock. L929 cells were grown and maintained in the complete EMEM medium and 2002 cells were grown in complete EBME medium, both at 37°C. The cultures were passaged weekly and media changed every two days. L929 cells in their 576th and 577th passages, and human fibroblasts in their 21st passage were used in this work.

Test method. On day 0, the test sugars were dissolved in the appropriate complete medium to give a concentration of 11 mM, and filter sterilized through an 0·2 μm Millipore filter. The morphology of the cells in the stock cultures was examined microscopically and the cell number defined using a Coulter Counter. Cells were then passaged into the appropriate media containing the test sugars. A haemacytometer count was used to confirm a single cell suspension. Replicate cultures for the assay were prepared by dispensing 1 mL of L929 cell suspension

Table 1. Effect of various monosaccharides on L929 and 2002 fibroblasts in culture.

	Percentage difference in cell yield relative to the control							
	Day 2		Day 4		Day 6		Day 8	
	L929	2002	L929	2002	L929	2002	L929	2002
Arabinose	12	7	3	3	−2	5	0	6
Fucose	−4	0	−7	1	−15	2	−24	3
Galactose	15	20	8	15	11	26	26	28
Glucose	−5	0	2	2	−3	0	1	4
Mannose	5	4	2	7	−4	7	−1	9
Rhamnose	6	1	2	5	−2	8	1	11
Xylose	8	9	5	4	4	3	11	3
Galacturonic acid	9	−3	−25	−12	−21	−11	−14	−12
Glucuronic acid	3	−9	−14	−15	−24	−17	−17	−14
Glucosamine	−29	−10	−46	−14	−48	−15	−50	−18
N-Acetylglucosamine	3	6	10	8	8	2	28	10

containing 1×10^5 cells mL^{-1} or 2002 cell suspension containing $1{\cdot}5 \times 10^5$ cells mL^{-1} into each well.

Identical control cultures in media not supplemented with a test sugar were also prepared. Test and control cultures were grown in a humidified incubator at 37°C in a 5% CO_2 in air atmosphere.

Haemacytometer counts were taken on days 2, 4, 6, and 8. Four wells from both test and control cultures were counted on each occasion as described previously (Turner et al 1989). On days 2, 4, and 6 the medium was changed for the appropriately supplemented complete medium in all wells.

Results and discussion

When compared with growth of cells in control cultures, those cultures supplemented with the simple sugars showed a variation in cell yield on days 2, 4, 6, and 8. The actual differences in cell yield between control cultures and cultures supplemented with the various sugars, expressed as a percentage, are shown in Table 1. Statistical analysis (Duncan's test for multiple comparisons) of the mean cell yield in test cultures on day 8 against control cultures was carried out. Percentage differences greater than about ±12% were found to be significant ($P < 0{\cdot}05$).

The observed effects could be grouped into three categories that could be related to the chemical structure of the monosaccharides. The acidic sugars galacturonic acid and glucuronic acid produced a marked reduction in the rate of growth of both human (Fig. 1, top) and L929 fibroblasts (Fig. 1, bottom). The inhibitory profile was the same for both cell lines. The cells showed no change during the first 24 h; in the following 6 days the growth rate declined, subseqeuently showing a recovery in the last two days relative to control cultures. Although no attempt was made to study this phenomenon in detail, we believe that it may be explained in terms of an initial enzyme inhibition event, followed by a recovery after the first six days in culture as a result of an adaption of the cells to the culture conditions.

The neutral sugars arabinose, glucose, mannose, and rhamnose failed to produce a significant change in the growth rate of either human (Fig. 2, top) or L929 cells (Fig. 2, bottom), whilst galactose, fucose, and xylose affected the cultures to a greater or lesser degree (Fig. 3). Xylose did not significantly affect the growth rate of human cells but increased cell yield of the L929 cells by 11% over control. Although a significant increase ($P = 0{\cdot}017$), the reason for the increase and the apparent species specificity remains unresolved. Galactose produced a marked and highly significant ($P = 0{\cdot}005$) increase in growth rate in both L929 and human fibroblasts which may be rationalized by a consideration of the literature on galactose metabolism. Christopher et al (1977) studied galactose metabolism in hamster NIL

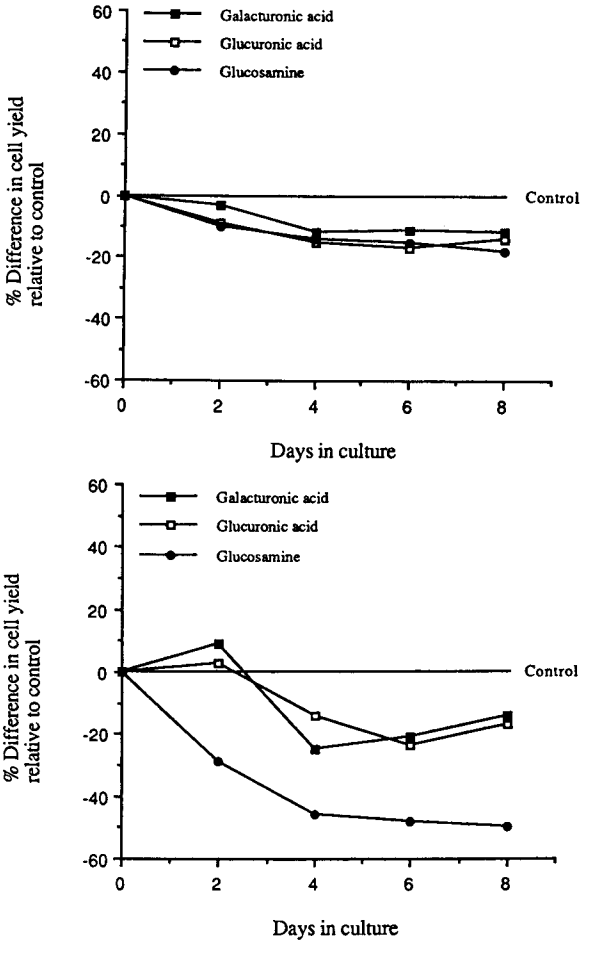

FIG. 1. Percentage difference in cell yield between control cultures and those exposed to galacturonic acid, glucuronic acid, and glucosamine. Human 2002 fibroblasts (top). Mouse L929 fibroblasts (bottom).

cells, and reported that the metabolism of galactose altered depending upon whether the cells were in a sugar-deprived (glucose or galactose) state or not. In glucose-fed cells, galactose was predominantly converted into nucleotide sugars such as uridine diphosphate galactose (UDP-Gal) whilst under glucose-starvation conditions an apparent metabolic block resulted in the accumulation of galactose-1-phosphate—a situation analogous to galactosaemia in man. In the presence of sufficient glucose, galactose and glucose are metabolically interchangeable

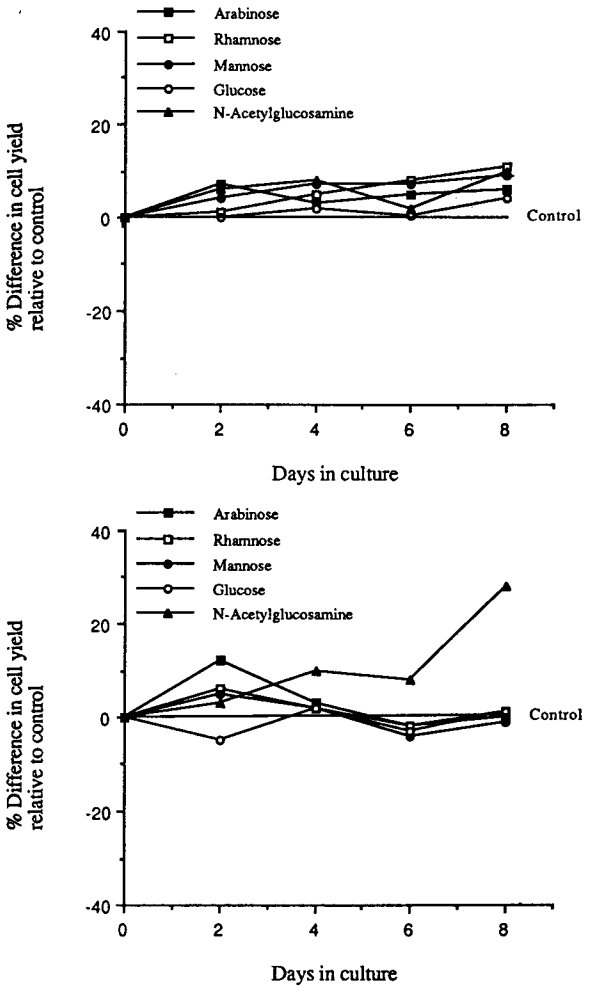

FIG. 2. Percentage difference in cell yield between control cultures and those exposed to arabinose, rhamnose, mannose, glucose, and *N*-acetylglucosamine. Human 2002 fibroblasts (top). Mouse L929 fibroblasts (bottom).

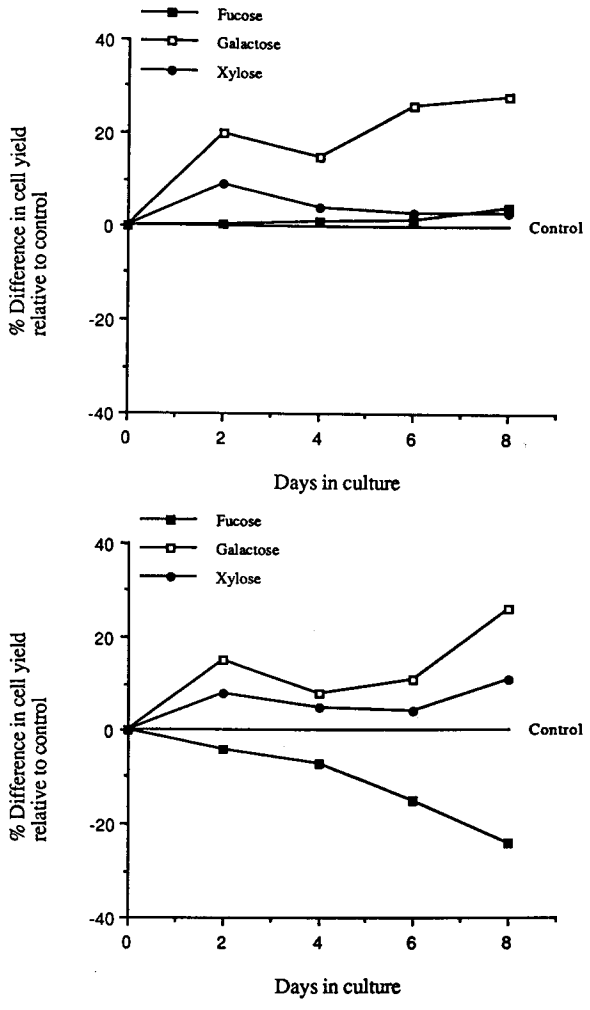

FIG. 3. Percentage difference in cell yield between control cultures and those exposed to fucose, galactose, and xylose. Human 2002 fibroblasts (top). Mouse L929 fibroblasts (bottom).

when attached to UDP (see Stryer 1981). If a similar metabolic process exists in the fibroblast cell lines used in this study, it is reasonable to conclude that galactose is a desirable though not essential nutrient. Thus, if sugar-starvation is a contributory factor in a non-healing wound, it may be predicted that the granulation process will be facilitated by increasing both glucose and galactose levels in the wound. Our results appear to lend support to this prediction.

Fucose produced a distinct species specific inhibition of growth. The growth of L929 cells was significantly inhibited ($P=0{\cdot}001$) whilst that of the human 2002 cells was not affected. In addition morphological changes such as rounding of the cells and loss of overlapping were observed. Morphological changes (elongation and flattening) have been reported for 3T3 (heteroploid embryonic mouse fibroblast) cells when L-fucose was added to the growing medium already containing normal amounts of glucose, yet no similar effect was observed in human diploid skin fibroblast cell lines (Cox & Gesner 1965). It was suggested that fucose induces alterations in the cell shape by binding to specific sites on the cell surface. Johnson & Schwartz (1976), who observed similar elongation and flattening with simple glucose deprivation, reinterpreted the observation, suggesting that L-fucose interferes with glucose metabolism and thus leads to decreased ATP levels. These earlier studies may well explain how fucose might inhibit cell growth but they do not, of course, shed any light on the species specificity observed. Interestingly, Bowness (1982) reported that wounded rat skin appears to incorporate fucose into a fucosylated glycoprotein.

The basic sugar, D-glucosamine produced marked inhibition in the growth of both cell lines which showed a different pattern from that observed in the cultures supplemented with the acidic sugars. Growth rate declined from the first day and the decline remained constant throughout the experiment. The recovery observed in the cultures supplemented with the acidic sugars during the last days in culture was absent in cultures supplemented with glucosamine (Fig. 1a, b). *N*-Acetyl-D-glucosamine, in which the basic group is masked, produced a significant ($P=0{\cdot}001$) final increase of 28% in the L929 cultures but the growth rate of the human fibroblasts was not significantly affected (see Fig. 2a, b). The reasons for these observations remain obscure.

In conclusion, our results demonstrate that whilst some simple sugars do appear to exhibit biological activity in L929 and 2002 fibroblast cultures, minor changes in the configuration of the hydroxyl groups and the presence of acidic or basic functional groups can result in either stimulation or inhibition of growth rate. If fibroblasts in healing wounds do respond to the presence of exogenous simple sugars in a similar way, we also suggest that an increased rate of granulation could be achieved in a wound through the appropriate choice of sugar as a healing aid.

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Suppression of pentobarbitone-induced hyperactivity by past experience in mice

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Abstract—Locomotor activity of CD-1 mice, tested in an unfamiliar environment (toggle-floor box), was increased either by a subhypnotic dose (20 mg kg^{-1}) of pentobarbitone or after recovery from pentobarbitone-induced (50 mg kg^{-1}) anaesthesia. On the contrary, when mice were tested 6 h after a single exposure to the apparatus, pentobarbitone in either case failed to produce hyperactivity. The results demonstrate that mice recovering from barbiturate anaesthesia maintain susceptibility to the exteroceptive stimuli provided by a novel environment and knowledge of the environment acquired during past experience.

Although generally regarded as sedatives, under some conditions, barbiturates may stimulate locomotor activity in laboratory animals (Stretch 1963; Harris et al 1966; Watzman et al 1968). Excitement induced by barbiturates is particularly interesting from the practical point of view as it is regarded as one of the unwanted but not uncommon side-effects of the drugs given as hypnotics (Harvey 1975). Moreover, such an excitement can be sometimes observed during recovery from barbiturate anaesthesia (Price 1975). We have recently observed that hyperactivity occurs not only in mice receiving subhypnotic doses of pentobarbitone (pentobarbital), but also in animals recovering from pentobarbitone-induced anaesthesia (Vetulani et al 1989). It is not known if the mechanism of these two kinds of hyperactivity is the same. The barbiturate-induced locomotor stimulation occurs when brain concentration of the drug approaches a particular level (Middaugh et al 1981) and this level can be reached after the administration of a suitable subhypnotic dose as well as during recovery from anaesthesia, when brain concentration of the barbiturate declines. Thus, it is likely that the same neurochemical mechanisms are responsible for both types of pentobarbitone-induced hyperactivity, but it cannot be excluded that the two barbiturate behavioural features might be differently influenced by the presence or absence of barbiturate metabolites as well as by environment and social factors.

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Since novelty of the environment is an important factor in the locomotor stimulatory action of drugs (Steinberg et al 1961; Rushton et al 1963; Oliverio & Castellano 1974), in the present study we investigated how a previous experience—the knowledge of an apparatus used for measurement of locomotor activity acquired during a single test carried out on naive, non drugged mice—affects the stimulatory action of a subhypnotic dose of pentobarbitone and posthypnotic hyperactivity.

Materials and methods

The subjects were naive male mice (27–32 g) of the randomly bred CD-1 strain (Charles River, Italy). Upon their arrival in the laboratory (7–10 days before the experiment), the mice were housed in standard transparent plastic cages (8 per cage), under standard laboratory conditions (free access to food and water, ambient temperature of 22°C, light on from 7 am to 7 pm). 48 h before the experiment, mice were placed in single 30 × 12 × 12 cm transparent plastic cages, where they remained till the test for locomotor activity.

The locomotor activity was measured in an apparatus consisting of 8 toggle-floor boxes, each divided into two 20 × 10 cm compartments connected by a 3 × 3 cm opening. For each mouse, the number of crossings from one compartment to the other was automatically recorded by means of a microswitch connected to the tilting floor of the box. The apparatus was located in a sound-insulated cubicle.

Inexperienced animals had never been in the toggle-floor box before the activity test. Experienced mice were placed for 30 min in the toggle-floor box, without treatment, 6 h before the activity test (this interval slightly varied in the case of the animals receiving the sleep-inducing dose of barbiturate). The treatment with a subhypnotic dose of pentobarbitone sodium (Clin-Midi, France; 20 mg kg^{-1}) took place 15 min before a 30 min activity test; the controls received saline solution (0·9% NaCl). Posthypnotic activity was measured, during 30 min, starting 15 min after the recovery of the righting reflex, in mice receiving 50 mg kg^{-1} pentobarbitone sodium, a dose which produced sleep lasting