drugs[10,22] cause the induction. In the local muscular burning injury, avidin induction is restricted to the injured area[10]. The ability of induced avidin to retain biotin in the tissues as an avidin-biotin complex might explain an increase in total biotin concentration in the chicken plasma, and red blood cells found in malaria parasite *(Plasmodium lophurae)* infection[23]. Green[24] has presented evidence for a genetic relationship between avidins and lysozymes. He states that if both avidin and lysozyme perform an antibacterial function, it is possible that these proteins will be produced together in situations where the organism requires protection against bacteria. If avidin is assumed to have a defensive function against micro-organisms, it is easy to understand its occurrence in the egg white, which contains many bacteriostatic or bactericidal proteins[6], and induction in bacteria-infected or injured chicken tissues.

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2 R. Hertz, Physiol. Rev. *26,* 479 (1946).
3 P.D. Jones and M.H. Briggs, Life Sci. *11,* 621 (1962).
4 V. Botte, S.J. Segal and S.S. Koide, Gen. comp. Endocr. *23,* 357 (1974).
5 H.A. Elo, M.S. Kulomaa and P.J. Tuohimaa, Comp. Biochem. Physiol. *62B,* 237 (1979).
6 R.G. Board and R. Fuller, Biol. Rev. *49,* 15 (1974).
7 N.M. Green, Adv. Protein Chem. *29,* 85 (1975).
8 B.W. Langer, Jr, and P. György, in: The Vitamins, 2nd edn, vol.2, p.352, Ed. W.H. Sebrell, Jr, and R.S. Harris. Academic Press, New York 1968.
9 R.E. Eakin, E.E. Snell and R.J. Williams, J. biol. Chem. *140,* 535 (1941).
10 H.A. Elo, M.S. Kulomaa and P.J. Tuohimaa, Acta endocr. *90,* 743 (1979).
11 M.S. Hofstad, B.W. Calnek, C.F. Helmboldt, W.M. Reid and H.W. Yoder, Jr, (ed.). Diseases of Poultry, 6th edn. The Iowa State University Press, Ames 1972.
12 M.S. Kulomaa, H.A. Elo and P.J. Tuohimaa, Biochem. J. *175,* 685 (1978).
13 R.D. Mandella, H.W. Meslar and H.B. White III, Biochem. J. *175,* 629 (1978).
14 H.W. Meslar, S.A. Camper and H.B. White III, J. biol. Chem. *253,* 6979 (1978).
15 D.H. Metz, Adv. Drug Res. *10,* 101 (1975).
16 E.F. Osserman, R.E. Canfield and S. Beychok (ed.). Lysozyme. Academic Press, New York 1974.
17 E.D. Weinberg, Microbiol. Rev. *42,* 45 (1978).
18 E.O. Stapley, J.M. Mata, I.M. Miller, T.C. Demny and H.B. Woodruff, Antimicrob. Ag. Chemother. *1963,* 20.
19 L. Chaiet, T.W. Miller, F. Tausig and F.J. Wolf, Antimicrob. Ag. Chemother. *1963,* 28.
20 E. Katchalski, M. Sela, H.I. Silman and A. Berger, in: The Proteins, 2nd edn, vol.2, p.405. Ed. H. Neurath. Academic Press, New York 1964.
21 M. Klockars and P. Roberts, Acta haemat. *55,* 289 (1976).
22 P.K. Heinonen, T. Metsä-Ketelä and P. Tuohimaa, J. Reprod. Fert. *52,* 159 (1978).
23 W. Trager, J. exp. Med. *77,* 557 (1943).
24 N.M. Green, Nature *217,* 254 (1968).

Inhibition of growth and lactic acid synthesis in *Lactobacillus casei* by maltol and its reversal by glutamic acid

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Summary. Maltol inhibited growth and lactic acid synthesis by *Lactobacillus casei.* The inhibition was partially overcome by the addition of casein hydrolysate and yeast extract. Some amino acid mixtures were also effective, among which glutamic acid was able to reverse the inhibition completely.

Introduction. Maltol is a product of the Maillard Reaction[2], a reaction occurring between carbohydrates and proteins or amino acids at elevated temperatures. The Browning Reaction (as it is also called) is found to occur commonly in milk products, bread, etc. In some systems, like microbiological growth media where carbohydrates and amino acids are usually added, the reaction takes place during heat sterilization. Similarly, sugar cane molasses, which is widely used as a raw material for industrial fermentation, also contains a large number of these brown products. Microbiological media containing these substances may not be suitable for a specified purpose as the micro-organisms may be affected by these substances, and this might change the normal course of their activity. It has been shown by

Table 1. Inhibition of growth and acid accumulation in *L. casei* culture by maltol and its reversal

Parameters studied	Concentration of maltol (mg/ml)	Percent inhibition over control* in parameters			
		Additions to growth medium			
		Nil	Tryptone 1 mg/ml	Yeast extract 1 mg/ml	Casein hydrolysate 1 mg/ml
Growth as turbidity	0.5	12	24	9.8	15
	1.0	25	36.3	11.1	16
	2.0	30	42.3	11.1	18
Total acids	0.5	16.6	16.6	0	0
	1.0	33	33.3	0	0
	2.0	40	36.6	4	0
Lactic acid	0.5	22.7	19.5	0	2.4
	1.0	34	23	0	0
	2.0	38.2	39	0	4.8

* Control is grown on medium alone.

various workers that Maillard Reaction products have growth-inhibitory as well as stimulatory action. Pashev[3] showed that furfural had a bactericidal action on 6 strains of *Blakeslea trispora*. In *Candida utilis* it affected the metabolism of the yeast[4,5]. Similarly, Yajima et al.[6] showed that an uncharacterized product of the Browning Reaction inhibited the growth of *Bacillus subtilis, Escherichia coli, Saccharomyces cerevisiae* and *Aspergillus niger*. Impens[7] reported the formation of a growth stimulatory factor for *Phycomyces blakesleanus* by heating glucose and glycine. In mammalian systems also these products were shown to have an inhibitory action[8]; the browned protein obtained after storing glucose with egg albumin was found to inhibit the growth of rats, other signs of toxicity had elevated levels: serum glucose, urea, alkaline phosphatase and aminotransferase. In the light of these reports a study was undertaken to investigate the effect of some Browning Reaction products on some fermenting micro-organisms. As a part of this study, in the present communication we report the effect of maltol on growth and acid production by *Lactobacillus casei* and the reversal of this effect by some amino acids.

Materials and methods. *Lactobacillus casei* NRC13013 (National Research Council, Canada) was grown on the medium of Kodicek and Mistry[9]. Tryptone and casein hydrolysate used for reversal were vitamin-free. All these crude extracts and amino acids were added to the medium before autoclaving and wherever necessary the pH was adjusted to its original value. Glucose was autoclaved separately and added aseptically after cooling. The inoculum was grown in 10 ml of medium for 20 h at 37 °C. The cells were centrifuged, washed and resuspended in 10 ml of normal saline. 0.1 ml of this suspension was used to inoculate 10 ml of the test medium. The tubes were incubated at 37 °C for 120 h under anaerobic conditions. At the end of incubation period growth was measured as turbidity with a Beckman spectrophotometer model B. Lactic acid in the fermented medium was determined by the method of Barker and Summerson[10]. Total acids were determined by titrating the fermented medium against standard alkali.

Results and discussion. The results presented are the mean value of 3 replicates in each experiment. Maltol inhibited growth as well as acid production by *L. casei*, over the range of 0.5 to 2 mg/ml (table 1). It was found that tryptone and yeast extract increased the growth and total acid production many times. Considerable amounts of acids other than lactic acid were formed in these cases in contrast to the control (medium alone), where lactic acid was apparently the only acid formed (data not presented). Inhibition of growth by maltol was augmented further by the presence of tryptone and it had almost no effect on the inhibition of acid production. Both yeast extract and casein hydrolysate partially reversed the growth inhibition and completely reversed the inhibition of acid production by maltol.

Lactobacillus casei is known to have specific amino acid requirements for its growth[11]. In view of the fact that casein hydrolysate and yeast extract, which are known to contain considerable amounts of amino acids, are able to reverse the inhibition caused by maltol, some amino acids were added exogenously to see if these amino acids could overcome the inhibition caused by maltol. Table 2 shows that mixture 1 (Glu. + Asn. + Ser. + Asp. + Gly.) and mixture 2 (Asp. + Glu. + Ser. + Gly.) were able to reverse partially maltol's effect on growth, whereas mixture 3 (Glu. + Ser. + Gly.) had almost no effect. But all 3 mixtures significantly reduced the inhibition of acid production by maltol. Asparagine and aspartic acid apparently contributed to the effect on growth and not to the effect on acid production, as their omission from mixture 3 did not cause much change in the degree of reversal as far as acid production was concerned. Further, the 3 amino acids of mixture 3 were added individually to see if any one of them was particularly responsible for decreasing the effect of maltol. Table 3 shows that glutamic acid alone or with glycine was quite effective in reversing the growth inhibition as well as the inhibition of acid production. Other combinations or individual amino acids could not reverse the growth inhibition at all, although inhibition of acid production was decreased by all the other combinations to varying degree. The presence of serine appears to decrease the effect of glutamic acid in reversing the growth inhibition.

The selection of these amino acids was based on considerable amount of literature available on the role of amino acids and peptides in the nutrition of *L. casei*. The requirement of strepogenin for the growth of *L. casei* was for the first time shown by Woolley[12], who found that a factor present in liver extract was required for the growth of *L. casei* and some other bacteria. Further work by Woolley[13] on strepogenin concentrates showed that strepogenin was peptide in nature. A number of synthetic peptides were tested by Woolley[14] and it was found that some peptides containing glutamic acid, serine and glycine showed strepogenin activity. Later work led to the discovery of several very highly active peptides, all of which contained glutamic acid (Woolley[11]).

The present findings suggest that maltol probably interferes with amino acid or peptide metabolism or uptake, and the addition of an excess of glutamic acid overcomes this inhibition. The role of glutamic acid in the intermediary metabolism serving as a link between carbohydrate and amino acid metabolisms is well known, and any interference with the metabolism of this key amino acid might very well affect the other related metabolic cycles and ultimately growth.

Table 2. Effect of amino acid mixtures on the inhibitory effect of maltol on *L. casei*

*Amino acid mixtures	Percent inhibition** in presence of 2 mg/ml of maltol		
	Turbidity	Total acids	Lactic acid
Nil	22.7	43.7	35.7
Mixture 1	15.3	3.3	6.8
Mixture 2	10.2	12.3	2.9
Mixture 3	19.2	5.0	7.4

* Mixture 1 contained Glu. + Asn. + Ser. + Asp. + Gly.; Mixture 2 contained Asp. + Glu. + Ser. + Gly.; Mixture 3 contained Glu. + Ser. + Gly. ** Percent inhibition in parameters was calculated with respect to control (grown on medium alone).

Table 3. Effect of glutamic acid, glycine and serine on the inhibitory effect of maltol on *L. casei*

Amino acids	Percent change over control* in presence of 2 mg/ml of maltol		
	Turbidity	Total acids	Lactic acid
Nil	77.3	56.3	64.3
Glu. + Gly. + Ser.	78.0	92.8	97.2
Glu. + Ser.	62.3	86.8	76.5
Glu. + Gly.	89.1	98.6	97.1
Gly. + Ser.	78.0	83.4	78.8
Gly.	68.7	83.4	83.8
Ser.	72.3	86.5	85.0
Glu.	100.0	96.8	120.7

*Control taken as 100. Glu.: glutamic acid, Gly.: glycine; Ser.: serine.

The results of our studies provide a wide scope for further investigations of the mechanism of interaction of maltol with amino acids. Further studies in this direction are in progress in our laboratory.

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2 L.C. Maillard, C. r. hebd. Séanc. Acad. Sci., Paris *154*, 66 (1912)
3 Il. Pashev, V. Kostov and L. Loseva, Mikrobiol. Inst. Bulg. Akad. Nauk. *21*, 1728 (1970).
4 L.A. Gusarova, Prikl. Biokhim. Microbiol. *6*, 509 (1970).
5 G.A. Soboleva, V.I. Golubkov and A.M. Vitrinskaya, Mikrobiologia *42*, 441 (1973).
6 M. Yajima, R. Ohta and S. Akatsuka, Japan Tokai *7461*, 313 (1974).
7 R. Impens, Bull. Soc. R. Bot. Belg. *106*, 39 (1973).
8 M. Tanaka, M. Kimiagar, T-C Lee and C.O. Chichester, Adv. exp. med. Biol. *86 B*, 321 (1977).
9 E. Kodicek and S.P. Mistry, Biochem. J. *51*, 108 (1952).
10 S.B. Barker and W.M. Summerson, J. biol. Chem. *138*, 538 (1941).
11 D.W. Woolley and M. Sprince, J. exp. Med. *80*, 213 (1944).
12 D.W. Woolley and M. Sprince, J. Am. chem. Soc. *67*, 1734 (1945).
13 D.W. Woolley, J. biol. Chem. *172*, 71 (1948).
14 D.W. Woolley, R.B. Merrifield, C. Ressler and V. du Vigneaud, Proc. Soc. exp. Biol. Med. *89*, 669 (1955).

Chromosome change during growth in *Puschkinia libanotica* L. (Liliaceae)[1]

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Summary. The variation in chromosome size in root-tip meristem of *Puschkinia libanotica* L. was studied at different days of root growth with special attention to DNA, RNA, total protein and histone contents. The results show that the size and organisation of chromosomes even within the one tissue is subject to considerable change during growth and development.

In *Puschkinia libanotica* L. we investigated chromosomal and cytochemical changes in root-tip meristems during the early stages of root growth following the sprouting of bulbs. Until recently, it has been generally accepted that the form and composition of chromosomes within cells of the same tissue remain constant. This work shows, in fact, variations in the chromosome 'phenotype' that are closely associated with growth and ageing.

Puschkinia libanotica L. has 5 pairs of chromosomes that are easily distinguishable. Bulbs were grown in distilled water under constant aeration. Roots were sampled at 6, 10, 14, 18, 22 and 26 days of root growth following sprouting of bulbs. 3 roots from each bulb were treated with 0.2% colchicine; fixed with aceto-alcohol (1:3); hydrolyzed in 1N HCl for 8 min and stained with feulgen. 5 metaphase cells were analyzed from each root-tip. Length and width of individual chromosome were measured by moving scale Vicker's micrometer. Chromosome volume was worked out considering chromatids as cylindrical. Nuclear volume $[\frac{4}{3}\pi \cdot \frac{1}{4}(\text{length} + \text{breadth})^3]$ was also estimated after isolating 2C nuclei from root-tip meristems at different days of root growth to record the variation in nuclear size. Nuclei were isolated following the method of Evans[3]. Total dry mass (TDM), nucleolar dry mass (NM) and chromosome mass (CM = TDM-NM) were measured from isolated 2C nuclei sampled at 10, 14, 18 and 22 days. Measurements were done by interference microscope as outlined by Davies[4]. This helps to determine whether change in chromosome size is due to a change in chromosome mass or due to merely a change in chromosome coiling and condensation. Further studies were undertaken to examine which of the chromosome components contribute to changes in chromosome 'phenotype'. For this purpose nuclear DNA, RNA, histone and total protein were estimated from freshly isolated 2C nuclei sampled at 10, 14, 18 and 22 days. The observations were recorded by Barr and Stroud microdensitometer at appropriate wave lengths (565 nm for DNA, 550 nm for RNA, 645 nm for histone and 400 nm for total protein). Staining procedures followed for DNA, RNA, histone and total protein were that of McLeish[5], Moss[6], Alfert and Geschwind[7] and Mitchell[8], respectively. Finally, mitotic index (MI) was calculated at different days of root growth. Results are presented in tables 1 and 2.

Measurements of metaphases show an increase in chromosome volume at 14 days, reaching a maximum at 18 days, and thereafter a drop to approximately the 14-day level. At

Table 1. Change in chromosome volume (CV), nuclear volume (NV), total dry mass (TDM), chromosome mass (CM), nucleolar dry mass (NM), and other nuclear characters at different ages of root growth in *Puschkinia*

Age in days	CV[a] (μm^3)	NV[b] (μm^3)	TDM[c] ($\times 10^{-11}$ g)	CM[d] ($\times 10^{-11}$ g)	NM[e] ($\times 10^{-11}$ g)	DNA[f] (arbitrary units)	RNA[g] (arbitrary units)	Histone[h] (arbitrary units)	Protein[i] (arbitrary units)	MI[k]
6	222.63	–	–	–	–	–	–	–	–	13.54
10	214.84	477.93	26.61	19.37	7.23	18.68	12.40	6.50	7.60	15.25
14	244.21	482.81	32.28	25.53	6.75	19.01	7.80	8.20	8.50	16.61
18	283.58	656.44	37.85	31.14	6.71	19.34	7.30	10.70	9.30	22.26
22	252.59	580.59	27.30	20.98	6.04	19.09	7.40	8.00	7.70	16.37
26	247.18	–	–	–	–	–	–	–	–	–
CD 5%	20.20	49.36	5.04	3.79	–	–	1.09	1.67	1.30	2.84

[a]Mean of 45 metaphase cells analysed from 9 root-tips taken from 3 bulbs. [b]Mean of 40 isolated 2C nuclei from 2 root-tips; each root-tip from a different bulb. [c,d,e]Mean of 40 2C nuclei isolated from 4 root-tips; 2 roots per bulb. [f]Mean values of nuclear DNA content of 20 2C nuclei isolated from 2 root-tips. [g]Mean nuclear RNA content of 40 2C nuclei isolated from 4 root-tips; 2 per bulb. [h]Mean nuclear histone values of 40 2C nuclei isolated from 4 root-tips; 2 per bulb. [i]Mean nuclear total protein values of 40 2C nuclei isolated from 4 root-tips; 2 per bulb. [k]Mitotic index calculated from 4 root-tips taken from 4 bulbs.