Technical Papers

The Influence of Liver *L. casei* Factor on Spontaneous Breast Cancer in Mice[1]

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In a previous publication (*3*) we reported that intravenous injections of crystalline *L. casei* factor led to complete regressions of spontaneous breast cancers in mice, in about one-third of the animals. We have been informed by Drs. Hutchings and Stokstad, of the Lederle Laboratories, that the correct tentative designation of this substance was fermentation *L. casei* factor. The isolation of this compound was announced, and its microbiological activity and other properties were described by Hutchings, *et al.* (*2*). More recently Angier, *et al.* (*1*) reported the synthesis of a compound identical with the *L. casei* factor from liver. This substance differs in microbiological activity from that of the fermentation *L. casei* factor used in our previously reported experiments in that the liver *L. casei* factor is about 17 times as active for the test organism, *Streptococcus lactis* R, at half maximum growth.

In the following communication we present evidence that the action of the liver *L. casei* factor is unlike that of the fermentation *L. casei* factor in therapeutic effect on spontaneous tumors in mice.

Ninety-eight mice bearing single spontaneous breast cancers were selected for these studies. In each case a definite diagnosis of malignancy was established by biopsy. The animals were kept on a normal diet (Rockland mouse pellets). Three groups were formed. The first group, 39 tumor mice, received 5 μg. of liver *L. casei* factor; the second group, 31 mice, received 100 μg. of the liver *L. casei* factor; and the third group, 28 mice, received 5 μg. of the crystalline fermentation *L. casei* factor. All substances were injected intravenously daily for a period of 4–6 weeks. As control, the data of 71 mice of the same strain, which were observed in the laboratory during a period prior to this experiment, were used.

The results are presented in Table 1. It is evident that the liver *L. casei* factor, administered intravenously in doses of 5 and 100 μg. was ineffective in producing regressions of these tumors. On the other hand, the fermentation *L. casei* factor led to complete regression of tumors in 11 of 28 mice, which is in agreement with previous observations made in our laboratory. It is interesting to note that while the control group and the group treated with 5 μg. of the liver *L. casei* factor had the same approximate mean life span, there was a significant increase in the incidence of lung metastases in the latter group. Of the group which received the 100-μg. dose of the liver *L. casei* factor, only 3 out of 30 mice showed lung metastases. This lowered incidence of lung metastases can best be understood by comparing the mean life span of this group with that of the group which received 5 μg. of the liver *L. casei* factor and with that of the control group. The mean life span of the group receiving the 100-μg. dose was reduced from 74

TABLE 1

Group	No. of mice	Substance and dose (μg.)	No. of mice with complete regressions of tumors	No. of mice with new tumors	No. of mice with lung metastases	Mean life span in days*	S.D.	Significance of difference
1	71	0	0	19	13 among 61	74 ± 6.2	51.9	Between Groups 1 and 3: 2.2
2	39	Liver *L. casei* factor (5 μg.)	1	12	19 among 32	75 ± 6	38.2	Between Groups 2 and 3: 2.4
3	31	Liver *L. casei* factor (100 μg.)	0	11	3 among 30	55 ± 6	33.5	.....
4	28	Fermentation *L. casei* factor (5 μg.)	11	2	†	After 100 days 23 mice alive	...	.....

* Life span calculated after start of experiment.
† An evaluation cannot be given since the majority of mice in this group are alive.

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to 55 days, and therefore the mice evidently did not live long enough to develop the lung metastases. The 3 mice which did show metastases at death lived 90 days. It was apparent during the experiment that the primary tumors of the mice receiving the 100-μg. doses of liver *L. casei* factor intravenously were growing much more rapidly than the tumors of untreated controls. Unfortunately, the wide fluctuations in the growth rate of these tumors do not allow any quantitative evaluation.

Whether these findings apply only to the particular strain of mice and method of assay used in these experiments needs further investigation. No conclusions should be drawn from these animal experiments as to the action of the liver *L. casei* factor on human cancer.

References

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Stability of Carotene in Dehydrated Carrots Impregnated With Antioxidants[1]

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Deterioration in quality of dehydrated carrots is usually associated with two different types of changes. One of these, resulting in a darkening of the carrots, occurs in an aqueous phase and is concerned with the union of amino acids and sugars—the so-called Maillard reaction. The other is made evident by the deterioration of the carotenoid pigment and is probably allied to the oxidative rancidity of the carrot oils. This report is concerned with the latter phase of deterioration.

We have shown in previous publications that, when carrots are dried, the carotene goes into solution in droplets of oil and that, upon storage, the carotene disappears, leaving colorless droplets which give a positive test for aldehydes with Schiff's reagent (*3*). It has been reported that carotene dissolved in oil hastens the oxidation of the oil (*1*). Furthermore, carotene is degraded concurrently with the oxidation of fats (*2*). These observations suggest that the oil droplets in the cells of dried carrots are undergoing oxidative changes and that the carotene is degraded as a result of these changes.

Blanching improves the stability of the carotenoids but does not stop their deterioration. Furthermore, leaching blanched carrots before storage accelerates the rate of pigment breakdown. (Compare Items 6, 7, and 8 with Item 15 in Table 1.) Tests with carrots which had been blanched but not dried showed that the rate of pigment breakdown could be retarded by the application of antioxidants (*4*). This paper reports the results of a survey of the action of some 30 tests of various antioxidants on blanched, dried carrots. The tests were carried out on carrots harvested during the summer.

Imperator carrots, about 4 months of age, were harvested during August, sliced (2–3 mm. thick), and blanched in steam for 5 minutes. The blanched slices were soaked for 5 minutes in solutions of various antioxidants. They were drained and dried in a tunnel dehydrator at approximately 60° C. to a moisture content of about 6 per cent and then ground on a Wiley mill to pass a 20-mesh screen, the smaller particles being sifted through a 48-mesh screen on a Rotap machine and discarded. The remaining grains were divided, half being stored in an incubator at 40° C., and half stored at room temperature. Both storage tests were carried out in darkness. Pigment concentration, in an acetone extract, was measured on the Evelyn colorimeter using the 440 filter.

TABLE 1

	Treatment	Carotenoids remaining in samples after storage at 40° C. (%)				
		48 hr.	72 hr.	96 hr.	120 hr.	168 hr.
(1)	.01% N.D.G.A.* + .3% $Na_2S_2O_5$ (in 40% ethanol) ..		95.8			91.5
(2)	.1% pyrogallol + .2% $Na_2S_2O_5$ ..	94.5				91.3
(3)	.1% ascorbic acid		101.0		93.0	89.5
(4)	.1% pyrogallol ..		94.5			85.1
(5)	.01% N.D.G.A. (in 40% ethanol) .		98.7		85.5	82.0
(6)	Blanch only		93.0		91.0	77.0
(7)	" "	94.0		92.0		75.0
(8)	" "	94.0		91.0		74.0
(9)	.1% piperidine ...	94.0		89.0		76.0
(10)	.2% pyrrole (in 40% ethanol) .	95.0		89.0		75.0
(11)	.1% hydroquinine	97.0				74.0
(12)	.2% lecithin (in 40% ethanol) .	94.0		87.0		71.0
(13)	Blanch	83.0		76.0		67.0
(14)	40% ethanol leach	93.0		77.0		57.0
(15)	Water leach	82.0		73.0		45.0

Other substances tested: hemin, tocopherol, tocopherol plus ascorbic acid, piperidine plus SO_2, phosphate buffer pH7, phosphate plus SO_2, diphenylamine, *p*-phenylene diamine dihydrochloride, N.D.G.A. plus ascorbic acid, gallic acid, maleic acid, oxalic acid, γ-tocopherol palmitate, and α-tocopherol succinate.

* N.D.G.A. = nordihydroguaiaretic acid.

The antioxidants tested are shown in Table 1. Since the procedure for impregnation of the antioxidants required a 5-minute soak in the solution of the antioxidant to be tested, standards for comparison were

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