# Effect of Vitamin E on Carcinogenicity of Methylcholanthrene.* (27918)

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Wheat germ oil, which is very rich in alpha tocopherol (Vit. E), is one of the dietary factors reported to affect the carcinogenic response. Jaffe(1) reduced the incidence of response to an intraperitoneal injection of methylcholanthrene more than 50% by adding wheat germ oil to the diet of mice. He did not believe that the results could be attributed to the alpha tocopherol, though this vitamin had been shown to reduce the toxic effects of methylcholanthrene. Haddow(2), as well as Cameron and Meltzer(3), have concluded that alpha tocopherol does not lessen the incidence of tumors from skin paintings with carcinogens. Rowntree *et al.* (4) fed a crude wheat germ oil to rats and guinea pigs without other carcinogens, and claimed to have produced sarcomas in the abdominal cavity with an induction period as short as 15 days. Others(5-9) were unable to confirm these findings.

There is, however, apparently no previous investigation of the effect of alpha tocopherol on subcutaneously injected methylcholanthrene. The purpose of this study was to ascertain whether oral administration of this vitamin exerted any effect on production of sarcomas by methylcholanthrene.

*Experimental data.* In the first experiment, 140 female mice of the C57 Leaden strain were divided into 2 equal groups, one of which had 0.67 g of alpha tocopherol added to each kilo of its Laboratory Chow diet–an increase of almost 100 times the normal content. The critical subgroup in each major group received a subcutaneous depot of methylcholanthrene in mineral oil (0.1 mg/ml) in the interscapular region.

None of the 70 control animals developed tumors in the 24 weeks of the experiment. Thirteen fibrosarcomas were found in the critical subgroup receiving a regular diet, while only 8 appeared in the critical subgroup fed a high Vit. E diet. This represents 54% and 30% respectively of those animals surviving after the appearance of the first tumor.

In the second experiment, 130 C57 Black male mice were divided into 2 equal groups, one of which had 7.0 g of alpha tocopherol added to each kilo of its Laboratory Chow diet. The two critical subgroups in each group received an interscapular subcutaneous depot of methylcholanthrene (0.1 mg/ml) dissolved either in tricaprylin or a solution of 10% Tween 60 and 90% tricaprylin.

In 26 weeks, 2 lymphomas appeared in the control animals, an incidence of 6%. The mice in the critical subgroups on a normal diet developed a total of 13 tumors (9 fibrosarcomas, 3 lymphomas, 1 hepatoma): an incidence of 33% after correction for early deaths. The critical subgroups on a high Vit. E diet had a total of 9 tumors (7 fibrosarcomas, 1 lymphoma, 1 squamous carcinoma): a corrected incidence of 21%.

*Discussion and conclusions.* The C57 mice have a low incidence of spontaneous tumors, but respond uniformly well to carcinogens. Age does not seem to be a prominent factor, though more primitive sarcomas are produced in very young mice(10). Mineral oil and tricaprylin, if critically purified, apparently have little or no specific influence on carcinogenesis when used as a vehicle in a subcutaneous depot. Alpha tocopherol appreciably inhibited the carcinogenic activity of methylcholanthrene in the first experiment, and failed to produce tumors in the controls. In the second experiment, which had a bascially similar plan, though differing in the sex and substrain of mice, dosage of Vit. E, and vehicle used for the carcinogen, inhibition of the carcinogenic activity of methylcholanthrene by alpha tocopherol was confirmed.

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The significance of these results approximates the 93% level(11,12).

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## Phenethylbiguanide and Triglyceride Synthesis.* (27919)

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The oral hypoglycemic agent, phenethylbiguanide (PEBG)‡ has been shown *in vitro* to increase the uptake of glucose by rat adipose tissue(1) and thus to have an effect similar to insulin. Unlike insulin, however, PEBG inhibits oxygen uptake by adipose tissue(2) and increases lactic acid production by rat diaphragm(3). PEBG, therefore, acts differently from insulin in certain respects, and the following experiments were done to ascertain its effects on triglyceride synthesis in isolated adipose tissue, a reaction shown to be increased by insulin(4).

*Methods. Intact tissue.* Sprague-Dawley rats, 200-300 g, were killed by a blow on the head and exsanguination. The epididymal fat pads were excised and portions, approximately 50-80 mg, were weighed and incubated in 3 ml Krebs-Ringer-Bicarbonate buffer, pH 7.4, containing 5 $\mu$M glucose, 2% crystalline bovine albumin, and 1-$C^{14}$-palmitic acid. Incubations were carried out for 3 hours in a Warburg apparatus under an atmosphere of 95% $O_2$-5% $CO_2$. Where oxygen uptakes were measured Krebs-Ringer-Phosphate buffer was used as the incubation medium and 100% $O_2$ as the atmosphere. PEBG was used in the concentrations noted in the figures.

Following incubation, the fat pads were removed, blotted and rinsed 3 times in 0.9% NaCl. They were homogenized with 10 ml of chloroform-methanol (2:1 volumes), filtered, and dried under nitrogen. Lipid fractionation was carried out as described below.

*Homogenates.* After removal from the rats, the epididymal fat pads were homogenized in KCl-Tris buffer, pH 7.4, using 200 mg tissue per ml of buffer. The homogenates were centrifuged at 800 $\times$ *g* for 20 minutes, and the middle layer, between the nuclear debris and the fat, was removed and filtered through cotton. Incubation flasks were then prepared in a manner similar to that of Steinberg, *et al.*(5) and held 2 ml of homogenate, 1 ml, $KH_2PO_4$ buffer, pH 7.4 containing 20 $\mu$M $PO_4$, 10 $\mu$M ATP, 2 $\mu$M $MgCl_2$, 10 $\mu$M $\alpha$-glycerophosphate and 0.08 $\mu$c sodium 1-$C^{14}$-palmitate. Incubations were carried out for 90 minutes in room air at 37°C. PEBG was used in the concentrations noted in the figures.

Following incubation, 2 ml of medium was removed, acidified, and extracted twice with 7 ml aliquots of petroleum ether (b.p. 30-60°C). These were pooled and dried under nitrogen. Lipid fractionation was carried out as described below.

*Fractionation of lipids.* The dried extracts were taken up in hexane and placed on a sili-

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