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Effect of Methyl Linoleate on Tissue Cholesterol of Normal and Vit. E-Deficient Rabbits.* (25410)

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It has been shown(1,2) that a diet high in polyunsaturated fatty acids could significantly lower serum cholesterol; whereas Vit. E-deficient diet increased serum and muscle cholesterol(3,4). Shull *et al.*(5) have shown that supplementation of Vit. E-deficient rations with certain antioxidants delayed onset of dystrophy symptoms, but did not prevent the rise in plasma and muscle cholesterol. It appears, therefore, that Vit. E and unsaturated fatty acids might be interrelated with one another in controlling cholesterol levels of various body tissues. Previous studies on the effect of Vit. E or essential fatty acids on serum or tissue cholesterol level have been made with either Vit. E or essential fatty acids alone. These have been further complicated by simultaneous feeding of cholesterol or other antioxidants. Our studies describe the effect of feeding Vit. E and methyl linoleate on cholesterol content of serum, liver, kidney, heart, spleen, brain and muscle of normal and Vit. E-deficient rabbits.

Methods. Forty-two rabbits of both sexes were divided into 4 groups and fed the following diets *ad lib.*: A. Dystrophy producing diet of Young and Dinning(6); B. Dystrophy producing diet plus 10% methyl linoleate; C. Dystrophy producing diet plus 8 mg oral alpha tocopherol acetate thrice weekly; D. Dystrophy producing diet plus 8 mg oral alpha tocopherol acetate thrice weekly plus 10% methyl linoleate. When methyl linoleate was incorporated into the diet, an equivalent weight of sucrose was deducted. After symptoms appeared(7), blood was withdrawn by heart puncture and animals were sacrificed by intravenous injection of air. Then aliquots of spleen, kidney, liver, heart, brain and muscle were removed, and approximately 100 mg (wet wt) were used for cholesterol analyses. Cholesterol was determined by the method of Herrmann(8) after slight modifications suggested by the author.

Results. Distribution of cholesterol in vari-

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TABLE I. Effect of Diet on Cholesterol Content of Rabbit Tissues. Values expressed as mg % cholesterol and probable error of mean.

Tissue	Diet A Vit. E defic.	Diet B Vit. E defic. + meth. lin.	Diet C Vit. E defic. + vit. E	Diet D Vit. E defic. + meth. lin. + vit. E
Serum	362 ± 19.7	230 ± 8.7	178 ± 9.4	147 ± 15.5
Spleen	426 ± 7.5	355 ± 12.2	388 ± 12.2	357 ± 7.8
Kidney	386 ± 11.2	328 ± 7.0	335 ± 11.4	346 ± 12.5
Liver	382 ± 15.3	294 ± 6.5	341 ± 24.9	343 ± 3.4
Heart	175 ± 5.4	142 ± 4.5	136 ± 4.9	147 ± 6.4
Brain	1531 ± 63.4	1509 ± 50.4	1406 ± 45.8	1580 ± 37.1
Muscle	173 ± 3.1	166 ± 5.1	71 ± 3.4	76 ± 9.9

ous tissues of normal and dystrophic rabbits is shown in Table I. Values are expressed as mg cholesterol/100 g wet tissue or 100 ml serum, and represent average of tissues from 10 animals each on Diets A & B, and 11 rabbits each on Diets C & D. There was a significant decrease in serum cholesterol ($p < 0.01$) of animals receiving dystrophic Diet B plus 10% methyl linoleate as compared to those on dystrophic Diet A alone. Less significant differences were noted in liver, heart, kidney and spleen ($p < 0.01$), while brain and muscle failed to show important variations in the 2 groups. When animals of Group A were compared to Group C, marked lowering in cholesterol of the latter group was noted in serum and muscle ($p < 0.01$) with less but significant difference in heart tissue ($p = 0.01$). The other tissues showed lower, but not significant differences. Tissue cholesterol of rabbits on Diet C did not vary from those receiving methyl linoleate on Diet D. Rabbits on Diet C had similar tissue cholesterol values to those receiving dystrophic Diet B supplemented with methyl linoleate. The exceptions were that serum cholesterol of the latter group had a slight increase ($p > 0.01 < 0.05$) while muscle cholesterol showed a marked elevation ($p < 0.01$).

Discussion. It has been previously shown (3,4) that diets deficient in Vit. E cause increased skeletal muscle and serum cholesterol, and our work substantiates these findings. Studies in several laboratories have established the effects of unsaturated fats in lowering serum cholesterol(1,2). Our data do not show that animals receiving Vit. E and 10% methyl linoleate have significantly lower serum or tissue cholesterol than animals receiving Vit. E alone. This may be explained by the fact that the latter group of animals did not receive an isocaloric substitution of hydrogenated oil as did those of other workers (9).

Perhaps the most important point from these experiments is that rabbits on a dystrophic diet with methyl linoleate had lower serum and tissue cholesterol than animals receiving the dystrophic ration alone. Only muscle and brain showed no decrease in cholesterol. Also rabbits fed methyl linoleate supplemented dystrophic diets had similar tissue cholesterol values to animals receiving Vit. E, muscle being the one exception. From these observations, it is concluded that Vit. E is a more important factor than methyl linoleate in maintaining normal tissue cholesterol metabolism, but methyl linoleate may aid in reducing tissue cholesterol, muscle excepted, when Vit. E stores are inadequate. It is conceivable that the beneficial effects of methyl linoleate in lowering tissue cholesterol may be due in part to the fact that it spares traces of Vit. E in the diet.

Summary. 1. Distribution of cholesterol in 7 tissues of normal and dystrophic rabbits was determined. 2. Significant decreases in serum, liver, heart, kidney, and spleen cholesterol occurred in animals receiving dystrophic diet plus 10% methyl linoleate as compared to those receiving dystrophic diet alone. 3. Vit. E caused a lowering of cholesterol in serum, muscle, and heart tissue. 4. 10% methyl linoleate did not significantly lower tissue cholesterol of normal animals. 5. Dystrophic rabbits receiving methyl linoleate had similar tissue cholesterol to normal rabbits with the exception of muscle tissue.

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Hormonal Augmentation of Antimetabolite Chemotherapy.* (25411)

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Our emphasis has been placed on utilizing combinations of drugs in treatment of neoplastic growth(1-5). Previous reports have shown mouse mammary adenocarcinoma 755 to be adversely affected by stilbestrol(5), and by the niacin antagonist, 6-aminonicotinamide (3). The present studies demonstrate the effects of the combined administration of the hormone and the anti-vitamin on the 755 tumor.

Materials and methods. Mammary adenocarcinoma 755 grown in the C57 Bl mouse was the tumor-host system used in all studies. The tumor was implanted into the right axillary region of the mouse by the usual trocar technic. The animals were 2-4 months old, weighed 18-22 g and were housed in plastic cages in an air-conditioned, constant temperature room (74°F). They had free access to Rockland pellets and water. 6-Aminonicotinamide (6-AN), in saline, was administered intraperitoneally at a dose of 2 mg/kg/day; stilbestrol and testosterone, in sesame oil, were administered intramuscularly at doses of 10 mg/kg/day and 25 mg/kg/day respectively. *Biological.* Treatment was initiated on very well-established tumors (14-20 days old) and was continued for 5 to 7 days. In order to note any delayed host toxicity, a waiting period of 5 to 6 days of no treatment was observed after last injection. At the end of this period the animals were sacrificed, the tumors were removed and wet weights were determined to the nearest milligram. A group of animals (labelled "sacrificed controls") was sacrificed on the day that treatment of the remaining groups was initiated. Mean tumor weight of this group served as a reference point for evaluating the effect of chemotherapy on other groups in the experiment. The statistical method used to calculate the significance of the results has been reported(3). *Enzymatic.* The animals were sacrificed by cervical rupture 24 hours after cessation of therapy. The tissues were immediately removed, chilled in crushed ice, blotted dry, and homogenized in 0.25 M sucrose containing 0.02 M nicotinamide and 0.004 M versene in a glass homogenizer of the Potter type(6). The ability of tissue homogenates to convert to citrate was measured as follows: pyruvate, 0.011 M; ATP, 0.007 M; $MgCl_2$, 0.008 M; nicotinamide, 0.04 M; potassium phosphate buffer (pH 7.4), 0.001 M; fluorocitrate, 2.15×10^{-4} M; and the appropriate volume of tissue homogenate was added to ice-cold reaction vessels. Ice-cold isotonic KCl was added to give, after addition of substrate, a final volume of 2.7 ml. Immediately before the vessels were placed in a constant-temperature water bath, 0.2 ml of a 0.10 M malate solution was added. All reactants were neutralized with KOH prior to addition. Incubation was carried out at 37°C with agitation for 30 minutes, at which time the reaction was stopped by addition of 0.5 ml of 30 trichloro-

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