TABLE V. Antiviral Activity of Tannin and Tannin-Free Fractions of Various Mint Plants in Herpes Simplex-Infected Eggs.

Plant	Virus dose (LD_{50}/0.3 ml)	Survivors/total eggs		
		Prep.*	Prep., no virus	Virus only
	Tannin fraction†			
Crea monda	25	5/9	5/5	1/4
Wild thyme	6	5/10	5/5	2/10
Marjoram	25	4/9	5/5	1/4
	Tannin-free fraction‡			
Crea monda	25	5/10	3/3	1/4
Wild thyme	6	8/10	5/5	2/10
Marjoram	25	0/4	4/4	1/4

* All plant preparations were injected 2 hr prior to virus.
† Tannin fractions from gelatin precipitation were diluted to original volume of aqueous extract and injected in 0.3 ml.
‡ Tannin-free preparations from gelatin precipitation were injected in 0.6 ml.

ponents but at concentrations too low to detect in antiviral tests.

Summary. Extracts of various plants of the mint family (Labiatae) were studied for antiviral activity. Peppermint *Mentha piperita* extract had antiviral activity against Newcastle disease (NDV), herpes simplex, vaccinia, Semliki Forest, and West Nile viruses in egg and cell-culture systems. It contains a tannin with an affinity for NDV and mumps virus and a nontannin fraction with antiviral effects against herpes simplex virus. Aqueous extracts of sage (*Salvia cyprea*), marjoram (*Origanum majorana*), wild thyme (*Thymus serpyllum*), American pennyroyal (*Hedeoma pulegioides*), Crea monda (*Satureia* sp.) and Spanish and French thymes (*Thymus* sp.) all exhibited some antiviral effects against NDV. The first 6 also exhibited some antiviral effects against herpes simplex. Hyssop (*Hyssopus officinalis*) extracts had activity against herpes simplex virus while rosemary (*Rosmarinus officinalis*), horehound (*Marrubium vulgare*), and catnip (*Nepeta cataria*) extracts were not detectably antiviral. None of these plant extracts produced an antiviral effect superior to that of melissa (*Melissa officinalis*) extracts. The data suggest the existence of some biochemical relationships among plants of the mint family, especially the presence of a common tannin.

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Influence of Cholesterol on Estrogen Induced Aortic Ruptures in Turkeys.* (31875)

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Aortic ruptures have been induced in turkeys using 2 synthetic compounds with estrogenic activity. The syndrome was produced when diethylstilbestrol (DES) was administered parenterally(1), or when incorporated in the feed(2). A high incidence of aortic rhexis has also been produced when dienes-

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trol diacetate (DD) was included in the diet(3).

Estradiol benzoate has been found to prevent coronary atherosclerosis in cholesterol fed cockerels(4). Estradiol valerate reduced the severity but not the prevalence of experimental coronary atherosclerosis in pigeons (5). However, an estrogen (2-iodo-estrone acetate) with little feminizing potency(6), and also an estrogen (doisynolic acid) with potent estrogenic effects(7) did not alter coronary atherosclerosis in cholesterol fed chickens. Therefore, the present study was initiated to determine whether dietary cholesterol had an influence on DES and DD induced aortic ruptures of turkeys.

Materials and methods. Broad-Breasted Bronze and Broad-Breasted White male poults were raised by conventional methods and fed a 20% protein commercial-chick-starter mash until 4 weeks of age. At this time they were changed to a 19% protein diet containing 0.5% NaCl(8). They were changed again at 6 weeks of age to a basal diet containing 23% protein, 6% fat, 3% NaCl, with variations of added supplements according to experimental groups.

Four separate trials were conducted. In each trial the turkeys were randomized into 11 groups of 8 birds each at 6 weeks of age. The following supplements were added to the basal diet and were fed to 2 groups of poults in each trial: 1) 1% added cholesterol, 2) 16 g DES†/100 lb of feed, 3) 1% added cholesterol and 16 g DES/100 lb of feed, 4) 102.4 g DD/100 lb of feed and 5) 1% cholesterol plus 102.4 g DD/100 lb of feed. These levels of DES and DD were used because they had been found previously to induce maximum mortality from aortic ruptures (3). One group of poults was fed unsupplemented basal diet and served as controls. Since 4 trials were conducted, a total of 8 groups were fed each supplemented diet and 4 groups the unsupplemented diet.

Indirect systolic blood pressure measurements(2) were made on 4 poults per group in each trial at 10 weeks of age. Total plasma lipid(9) and cholesterol(10) values were determined on 4 poults per group in each trial at 11 weeks of age. The experiment was terminated when the turkeys were 11½ weeks of age. At this time, frozen sections of myocardium containing branches of the left coronary artery and sections of the abdominal aorta of treated and control turkeys were prepared and stained with hematoxylin-eosin and Oil red O-luxol fast blue stains.

Results. There were no significant interactions between trials and treatments, therefore the results of the 4 trials were combined. No deaths due to aortic rupture occurred among the control or cholesterol fed birds; however, 37% of the poults fed DES died, and 6% of those fed DES and cholesterol succumbed (Table I). Forty percent of the turkeys fed DD died, while 47% of the poults fed DD and cholesterol died from aortic ruptures.

Supplementation of the basal diet with cholesterol caused a significant lowering of systolic blood pressure (Table I). However, this reduction in blood pressure was not as great as that obtained from feeding of DES or DD, and the 2 hormones were equally effective in reducing blood pressure. Addition of cholesterol to diets containing DES or DD did not influence systolic blood pressure.

Coronary atherosclerosis was observed in frozen sections of myocardium of all groups of birds except controls. Atherosclerotic lesions, with medial necrosis, were most severe in the DES, DD, and DD-cholesterol fed poults (Fig. 1). Moderately severe lesions without medial necrosis, were present in DES-cholesterol fed turkeys, and there was mild coronary atherosclerosis in cholesterol fed birds. Atherosclerosis was characterized by lipid deposition in the tunicae media and intima of involved coronary arteries along with proliferation of the tunica intima and fragmentation of the internal elastic membrane.

Atherosclerosis of the abdominal aorta was equally severe in DES, DES-cholesterol, DD, and DD-cholesterol fed turkeys. Less severe lesions were seen in cholesterol fed turkeys, and occasional mild atherosclerotic lesions

† Stilbosol, Eli Lilly Co., Greenfield, Ind., contained 20 g/lb in a carrier of polyethylene glycol 200.

were observed in control poults. There was lipid deposition in the tunicae intima and media of aortas of all birds along with proliferation of the tunica intima and rupture of the internal elastic membrane.

Discussion. Estrogens have been reported to prevent coronary atherosclerosis and reverse the previously induced disease in cholesterol fed cockerels, but to have no effect on aortic atherosclerosis(4,11). In the present study, the addition of cholesterol to a DES supplemented diet markedly reduced the incidence of aortic ruptures induced by levels of DES previously reported to have feminizing effects(12). It has been postulated that the protective and therapeutic effects of estrogens against coronary atherosclerosis in chicks were related to plasma cholesterol/phospholipid (C/P) ratios, but it was not known whether this relationship was a direct result of estrogen treatment(13). In pigeons, elevation of phospholipids was not associated with spontaneous coronary atherosclerosis (14).

Total plasma lipid and cholesterol levels were highest in groups of turkeys in which greatest mortality occurred (Table II). Moreover, the total plasma lipid/total plasma cholesterol ratio was also highest for these groups. The total plasma lipid/total plasma cholesterol ratio was 3.76/1 for control birds and 1.71/1 for cholesterol fed turkeys. The ratio was 12.99/1 for DES fed birds and 2.81/1 for DES and cholesterol fed poults. Dienestrol diacetate-fed turkeys had a ratio of 14.17/1 and the ratio for DD and cholesterol fed poults was 8.46/1.

It appeared that the protection from aortic rhexis afforded to turkeys by addition of dietary cholesterol to a DES fortified diet resulted from the lowering of total plasma lipid and possibly from reduction of total plasma lipid/total cholesterol ratios of DES-cholesterol-fed poults, as compared with DES-fed birds. It is possible that the addition of cholesterol to the DES supplemented diet interfered, in some unexplained way, with the ability of DES to produce hyperlipemia, and therefore reduced its rhexis inducing potential. As described here and previously(15), DES induced aortic ruptures apparently resulted from previous development of aortic atherosclerosis in the area of the aorta that subsequently ruptured. On the other hand, it appeared that the effect of DD was not altered when cholesterol was added to the diet because similar mortality and hyperlipemia resulted from feeding of DD and DD-cholesterol supplemented diets.

TABLE I. Mortality and Blood Pressure of Turkeys Fed Estrogens and Cholesterol.

Treatment	Systolic blood pressure* (mm/Hg)†	% Mortality†
0	224[a]	0[b]
Cholesterol	200[b]	0[b]
DES	175[c]	37[a]
DES–cholesterol	178[c]	6[b]
DD	173[c]	40[a]
DD–cholesterol	174[c]	47[a]

* At 10 weeks of age.

† Means with different superscripts are significantly different according to Duncan's Multiple Range Test.

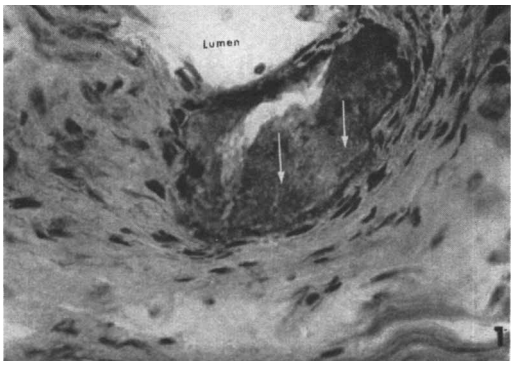

Fig. 1. Necrosis and lipid (arrow) in the wall of a coronary artery of a poult treated with DES. H & E X 400.

Summary. A high incidence of aortic ruptures occurred when turkeys were fed diets containing diethylstilbestrol, dienestrol diacetate, and dienestrol diacetate-cholesterol. A low incidence of mortality occurred when turkeys were fed a diet fortified with both cholesterol and diethylstilbestrol, and there was a marked lowering of total plasma lipid of such treated poults. No mortality occurred in cholesterol fed and control birds. Coronary atherosclerosis was severe in diethylstilbestrol, dienestrol diacetate, and dienestrol diacetate-cholesterol fed turkeys, moderately severe in diethylstilbestrol-cholesterol fed turkeys, and mild in cholesterol fed turkeys.

TABLE II. Plasma Lipid and Cholesterol Levels of Turkeys Fed Estrogens and Cholesterol.

Treatment	Total lipid* (mg/100 ml)	Total cholesterol* (mg/100 ml)	Total lipid/total cholesterol
0	691 ± 189	184 ± 28	3.76/1
Cholesterol	1,112 ± 359	648 ± 166	1.71/1
DES	13,839 ± 3,095	1,065 ± 266	12.99/1
DES–cholesterol	2,167 ± 660	770 ± 228	2.81/1
DD	16,103 ± 1,390	1,136 ± 261	14.17/1
DD–cholesterol	16,662 ± 3,850	1,967 ± 608	8.46/1

* Mean ± standard error at 11 weeks of age.

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Experimental Studies of Factors Influencing Hepatic Metastases. XVIII. Significance of Trapped Tumor Cells.* (31876)

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Partial hepatectomy(1), vena cava constriction(2), reticuloendothelial system blockade(3), mechanical trauma(4), high fat diet (5) and dextran(6) have been observed to increase the incidence as well as size of hepatic metastases following intraportal injection of relatively known numbers of Walker tumor cells in Sprague-Dawley rats. Precise mechanisms responsible for the enhanced metastases have not been elucidated, although evidence suggested that this effect resulted from hepatic damage and/or the trapping of tumor cells within the liver. The present study was performed with ^{51}Cr-labeled tumor cells to determine the significance of increased retention of tumor cells in this regard.

Material and methods. Adult female Sprague-Dawley rats,† weighing approximately 200 g, were employed in all experiments. They were housed in individual cages and, except for those receiving a high fat diet, were permitted Purina Laboratory Chow and tap water *ad libitum*. Animals on the high fat diet were fed a 30% fat, choline-free diet for 4 weeks before tumor cell injection. Their controls were pair-fed with a 3% fat diet during this period.

The Walker carcinoma, propagated in this

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† Holtzman Farms, Madison, Wis.