tained in the presence of insecticides for periods up to 108 days. At certain intervals the treated cells were subcultured without insecticides and then infected with poliovirus or submitted to reaction with diphtheria toxin. The Cygon-, Dipterex-, DI-Syston-, chlordane-, and Karathane-treated cultures were more susceptible to poliovirus infection and the infected cells degenerated more rapidly. The malathion-treated cells did not show any change in susceptibility. The Karathane-treated cells were 10 times more resistant to the lethal action of diphtheria toxin than the control. Other treated cultures showed no changes with respect to diphtheria toxin reactivity. The results indicate some alterations in physiology of the insecticide-treated cells which affect the cell response to 2 other biologically active agents—poliovirus and diphtheria toxin.

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Dietary Saturated Medium-Chain Triglycerides and Vitamin E Deficiency in Chicks and Rats.* (30478)

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In the course of a study of the effects of dietary saturated medium-chain triglycerides (C_6-C_{12}; henceforth MCT) on serum cholesterol levels, it was noted that these triglycerides had opposite effects on chickens and rats. The levels in the latter were lower, those in the former higher than in controls(1).

During these studies exudative diathesis was occasionally noted in the chickens fed MCT. This was remarkable because the basic diet contained some added alpha tocopherol, and more than twice the amount of soybean meal, which had been found by Nesheim and Scott(2) to be effective against the occurrence of exudative diathesis; also, the diet was low in polyenoic acid, feeding of which has been shown to promote exudative diathesis by Dam *et al*(3). In view of the fact that exudative diathesis is one of the main symptoms of vit E deficiency in chickens, we decided to study the influence of MCT on the deficiency state in this species and to contrast its effects with those of tocopherol-deficient rats. Such a study seemed interesting not only because of the influence of MCT on cholesterol metabolism but also because MCT has been shown to be capable of counteracting some nutritionally induced disease states in the rat, particularly the consequences of essential fatty acid deficiency(4).

Materials and methods. Chickens: Groups of 15 to 25 male Leghorn or Columbian-cross chicks (♀ × New Hampshire ♂) were placed on the experimental diets at one day of age for periods of 4 weeks. The fat supple-

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ments used included MCT,† tricaprylate‡ and tricaprin,‡ all of which were prepared as previously described(1); triolein (technical grade), which contained 65.4% oleic acid and 8.3 linoleic acid as one of several contaminating fatty acids (our analysis), corn oil,§ and oleic acid (99%+ purity). Alpha tocopherol, when used, was added as the succinate at the rate of 2.5 mg/kg diet, and selenium, when used, was added as the dioxide at the rate of 1 mg/kg diet. The basic diet had the following composition (in %): soybean oil meal (50% protein), 40.0; fiber, 5.0; dicalcium phosphate, 2.0; trace mineral concentrate,‖ 1.0; sodium chloride, 0.5; DL-methionine, 0.2; choline chloride (70% concentrate), 0.3; vitamin E-free vitamin mix, 0.2; and glucose monohydrate to 100. At the end of each experiment, a number of chicks from a control group as well as those with gross overt symptoms of exudative diathesis were killed with chloroform and tissues from various organs placed in formalin solution for later histological examination. Alpha tocopherol determinations were made¶ on composites of 5 livers for certain groups and on pooled whole egg samples by the method of Pudelkiewicz *et al*(5).

Rats: The effects of MCT were compared with those of saturated triglycerides of longer chain length (C_{12-18}; henceforth LTC). The tocopherol content of the triglycerides was determined by the method of Kaunitz and Beaver(6) and found to be below 1 mg per 100 g. The triglycerides were fed in a purified diet containing 30% alcohol washed casein, 54% glucose, 2% cellulose, 3.5% USP salt mixture XIII, 0.5% calcium carbonate, 20% triglycerides, 1% linoleic acid and vitamins as reported previously(4) but with tocopherol omitted. Control groups were given by dropper 2 drops twice weekly of alpha tocopherol acetate.

† Generously supplied by E. F. Drew & Co., Boonton, N. J. Fatty acid composition, in %: caprylic, 70; capric, 25; C_6 and C_{12}, 5.

‡ Generously supplied by E. F. Drew & Co., Boonton, N. J.

§ Staley's salad oil.

‖ Composition, in %: calcium, 32.0; magnesium, 2.6; fluorine, 0.01; manganese, 1.0; iron, 0.175; iodine, 0.225; copper, 0.125; zinc, 0.009; and cobalt, 0.010.

¶ We would like to thank Dr. L. D. Matterson, Univ. of Connecticut, Storrs, for these analyses.

TABLE I. Effect of Various Dietary Lipids on Growth and Incidence of Exudative Diathesis.

Dietary lipid	Breed	4-wk body wt, g	Exudative diathesis incidence
Corn oil, 10%	Leghorn	293 ± 6*	0/24
MCT, 10%	"	224 ± 9	19/25
Corn oil, 10%	Columbian cross	387 ± 11	0/20
MCT, 10%	Columbian cross	325 ± 10	0/20

* Mean ± S.E.

Matching groups of weanling male rats of the Columbia Sherman strain were started on the diets. Their mothers had been placed, toward the end of gestation, on a triglyceride-free diet similar to the experimental ration but with glucose replacing the fat; no tocopherol was given to the mothers; the controls were given the first supplements after weaning. Weights were taken at least once weekly; the animals were sacrificed after they had consumed the experimental diets for 58 days, at which time they were 84 days old. Then organs were weighed and their testes fixed in Bouin's solution for H and E staining.

Results. Chickens: Table I shows the high incidence of exudative diathesis observed in Leghorn chicks receiving 10% MCT in their diets. Although the Columbian-cross chicks exhibited the same growth retardation as the Leghorns on the MCT diet, there were no vit E deficiency symptoms. In the second experiment (Table II), both tricaprylate and

TABLE II. Effect of Various Dietary Lipids on Growth and Incidence of Exudative Diathesis of Leghorn Chicks.

Dietary lipid	4-wk body wt, g	Exudative diathesis incidence	Mortality*
MCT, 10%	203 ± 11†	9/14	1/15
Tricaprin, 10%	197 ± 16	10/10	5/15
Tricaprylate, 10%	212 ± 12	7/12	3/15
Triolein, 10%‡	264 ± 11	6/15	0/15

* The chicks that died showed typical symptoms of exudative diathesis.

† Mean ± S.E.

‡ Technical grade, containing 65.4% oleic acid and 8.3% linoleic acid by GLC analysis in our laboratory.

TABLE III. Effect of Various Diet Supplements on Growth and Incidence of Exudative Diathesis.

Dietary supplement	Breed	4-wk body wt, g	Exudative diathesis incidence
None	Leghorn	277 ± 9*	0/15
"	Columbian cross	426 ± 11	0/15
MCT, 10%	Leghorn	238 ± 14	5/15
" "	Columbian cross	391 ± 11	0/15
Oleic acid, 10%†	Leghorn	292 ± 8	0/15
MCT, 10% +			
Selenium dioxide, 1 mg/kg	"	238 ± 7	0/15
α-Tocopherol succinate, 2.5 mg/kg	"	250 ± 9	0/15

* Mean ± S.E. † 99%+ pure.

tricaprin produced a growth depression and exudative diathesis, although the tricaprin was more potent in this regard. To a lesser degree, triolein supplementation also gave rise to exudative diathesis, probably due to the high contamination by linoleic acid. Results of the third experiment (Table III) showed that MCT but not pure oleic acid nor the lipid-unsupplemented, vit E-low diet produced exudative diathesis in the Leghorn chicks. As in the first experiment (Table I) the Columbian-cross chicks did not show any deficiency symptoms. Additions of selenium or of alpha tocopherol to the MCT-containing diet prevented all signs of exudative diathesis.

Tocopherol analysis showed the livers of MCT-supplemented or unsupplemented birds to contain less than 1 μg per g wet liver tissue. Tocopherol analysis of eggs from both breeds studied showed 41 and 51 μg per g fresh egg for the Leghorns and Columbian-crosses, respectively.

Histological examination of tissues showed no lesions of any kind in the control birds. For the birds with overt symptoms of exudative diathesis as a result of MCT, tricaprin, or tricaprylate addition, lesions were founds in the voluntary muscles. These showed hemorrhages, edema, vascular congestion, early focal degeneration, Zenker-like degeneration, and sarcolemmal proliferation. The histological changes were typical of exudative diathesis as described originally by Dam and Glavind(7).

Rats: When the animals were sacrificed, the average weights of the control and deficient groups ranged from 230 to 262 g; the differences were not significant.

Testicular weights and state of degenerative signs, classified according to Mason(8), are given in Table IV. The testes of the rats fed MCT were, on the average, not significantly lighter than those of the controls. Those fed LCT had testes weighing significantly less than any other group. Histological examinations confirmed the weight difference between the 2 vit E-deficient groups. The severity of the lesions was evaluated on

TABLE IV. Testicular Weight and Histological Appearance of Testes in Rats Fed a Vitamin E-Low Diet Supplemented with 20% MCT or LCT.

	Testicular weight			
	MCT		LCT	
	No. of rats	Testicular wt, g	No. of rats	Testicular wt, g
Controls (+ alpha tocopherol acetate)	8	2.8 ± .14*	8	2.8 ± .16
Vitamin E-deficient	11	2.6 ± .07	12	1.8 ± .13

	Testicular degeneration					
		State of testicular degeneration†				
Vit. E-deficient diet containing	Normal	I	II	III	IV	V
MCT	8	3				
LCT	5	1	1	1	2	2

* Mean ± S.E.

† Classified according to Mason(8); there were no abnormal histological findings in the testes of the control animals given alpha tocopherol acetate.

coded slides by Dr. Karl Mason. Only 3 of the 11 rats fed MCT showed mild degenerative changes whereas 6 of the 12 animals fed LCT had advanced lesions.

Discussion. Vitamin E deficiency symptoms vary widely not only among species but also within the same species. In rats, for instance, males characteristically develop testicular degeneration(8), while pregnant females given a diet containing oxidized lipid (9) develop the generalized Shwartzman reaction(10). For chicks, exudative diathesis is produced under some conditions, while encephalomalacia occurs under others. In the present study we even observed a marked difference in the manifestations of exudative diathesis between Columbian-cross and Leghorn chickens. It is uncertain whether hybrid vigor or differences in breed was responsible for this finding. Howes and Hutt showed a breed difference in susceptibility to encephalomalacia wherein 2 heavy breeds were more susceptible than the White Leghorns(11). To account for the variability it has been suggested that vit E may affect all parts of the cell and that the development of a particular symptom may be an expression of the site within the deficient cell damaged by the specific stress. The part played by vit E in cellular metabolism is still controversial; many believe that the effect of this vitamin is based on its capacity to prevent uncontrolled free radical formation at the site of stress(12,13). Others are inclined to ascribe to it a role in an essential enzyme system(14).

One wonders whether these considerations can help in clarification of the disparate observations that testicular degeneration in the vit E-deficient rat is beneficially influenced by the feeding of MCT whereas the tendency of at least one breed of vit E-deficient chicks to develop exudative diathesis is aggravated by its administration. The difficulty of finding such a common mechanism is evident from the dissimilarity of the two conditions as regards dietary selenium. The symptoms in the chick can be prevented but those in the rat are not influenced by inclusion in the diet of selenite(14).

It seems to us that the relation of vit E deficiency to linoleate metabolism may form the missing link. In rats, MCT has been shown to decrease linoleate requirements(4); therefore, one can speculate that MCT feeding is associated with less linoleate turnover than LCT feeding. Thus, the chances for uncontrolled free radical formation may be relatively smaller in an MCT-fed, vit E-deficient rat, giving protection to the target organ.

In view of the fact that the effects of MCT on serum cholesterol are opposite in the chicken and the rat, one could speculate that MCT feeding also has opposite effects on linoleate metabolism. Thus, MCT feeding may increase linoleate requirement in the chicken and thereby lead to more tissue peroxidation associated with a higher incidence of exudative diathesis.

Summary. Saturated medium-chain triglycerides (C_{6-12}; MCT) were included in vitamin E-deficient diets fed to chickens and rats. This produced a high incidence of exudative diathesis in the birds but exerted a protective effect on testicular degeneration in the rats. It is speculated that this difference is related to the capacity of dietary MCT to decrease linoleate turnover in the rat but to increase it in the chicken, leading to opposite effects in the affected organs of the two deficient species.

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Prevention of Traumatic Bleeding by Ellagic Acid in Rats.* (30479)

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Ellagic acid activates Hageman factor *in vitro* and *in vivo*(1,2) and causes a state of hypercoagulability in several animal species(2,3). This state of hypercoagulability is characterized by slight shortening of the glass clotting time and marked shortening of the silicone clotting time(2,3), increased prothrombin consumption(2), shortened partial thromboplastin time(2), and acceleration of thromboplastin generation(2,3). It was also noted that normal dogs, rabbits and cats when treated with ellagic acid showed decreased oozing from raw surfaces and from the site of venipuncture when compared with controls(3).

The purpose of this study was to evaluate the effect of ellagic acid on bleeding in the rat as determined by surgical removal of the terminal part of the tail.

Materials and methods. Female white rats (103), Carworth Farms, 350 to 500 g, were maintained under the same conditions of diet (Purina Chow) and environment.

Ellagic acid (4.4′, 5.5′, 6.6′ hexahydroxydiphenic acid 2.6, 2′6′ dilactone) (K & K Lab., Jamaica, N.Y.): dissolved in 0.025M sodium barbital buffer in 0.125M sodium chloride (pH 7.5). A solution with a concentration of 2×10^{-4}M was used.

Procedure. Anesthesia: Intraperitoneal sodium pentobarbital. 15 mg/animal.

The femoral vein was exposed surgically for injection of solutions. Fifty-three animals were injected with 1 ml of the ellagic acid solution per 100 g of body weight in 5 minutes. Fifty animals (controls) were injected with an equal amount of buffer in 5 minutes.

Immediately after the administration of ellagic acid or buffer the tail of the animal was amputated 5 cm from the tip. The animals were then placed on a flat surface with the tails hanging down into a test tube containing 5 mg of EDTA.

The amount of blood loss was measured 30 minutes after the tail wound had stopped bleeding. All animals were then kept under observation for 2 weeks for possible recurrence of bleeding from the site of amputation.

Results. The average blood loss observed in control animals was 4.4 ml while the average blood loss in ellagic acid treated was 0.3 ml. The distribution of the single values of blood loss and the means obtained in the 2 groups of animals are shown in Fig. 1. The difference between the 2 means was highly significant: ($t = 18.6$, $P<0.001$).

There was minimal overlap in the amount of blood loss. All control animals lost more than 1 ml of blood. Only 4 of the ellagic acid treated animals lost more than 0.9 ml of blood. In every case the blood loss with ellagic acid was well below the mean blood loss of the control animals (Fig. 1). In 14 of the 53 ellagic acid treated animals there was no blood loss, *i.e.*, not one drop of blood from the cut tail.

The duration of bleeding was 0 to 10 minutes in ellagic acid treated animals and 10 to 45 minutes in the controls.

No recurrence of bleeding was observed in any ellagic acid treated animal once the

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