turnal hemoglobinuria (PNHE) is intensified by reducing the ionic strength to 0.042. The ability of PNHE to form an intermediate complex by direct reaction with isolated C'3a ($PNHEC'_{3a}$) is not affected by decreasing the ionic strength; the hemolysis of $PNHEC'_{3a}$ is inhibited by reduction in ionic strength. The effects of low ionic strength and first complement component (C'1) esterase or a C'1 activator upon the enhancement of PNHE lysis are not additive, which suggests that these factors share a common mechanism. The effects of ionic strength on PNHE hemolysis are similar to those observed in other complement dependent hemolytic systems.

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Extent of Tocopherol Depletion *Versus* Onset of Creatinuria in Rats Fed Saturated or Unsaturated Fat.* (30635)

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The rate of production of a specific tocopherol-deficiency sign in the rat, nutritional muscular dystrophy, has been related to the fatty acid composition of the diet and of the tissues(1-5). Onset of nutritional muscular dystrophy in the rat is marked by a rather sudden, drastic increase in the excretion of creatine in the urine and is therefore readily detected.

The present experiment was undertaken to explore the interrelations between tissue α-tocopherol levels, fatty acid composition, and *in vivo* lipid peroxidation.

Experimental. Male weanling rats of the Sprague-Dawley strain were fed a tocopherol-deficient diet containing 23.7% casein, 58.2% cerelose, 4.9% salts 446(6), vitamins(7) made up in cerelose 0.7%, and 12.5% fat specially prepared to remove tocopherol(1). The diet contained 0.04 ppm of selenium as determined by neutron activation analysis.† Groups 1 & 2 were fed a tocopherol-free (<0.25 μg/g) fat containing 6.2% saturated, 8.2% monoenoic, 5.9% dienoic, and 23.7% trienoic acids, and having an iodine value(8) of 82.0. Groups 3 & 4 were fed tocopherol-free (<0.1 μg/g) coconut oil. Groups 2 & 4 received oral supplements of *d*-α-tocopheryl acetate 3 times a week.

Rats were sacrificed by dislocation of the cervical vertebrae. Small samples of the gastrocnemius and quadriceps muscle were excised for fatty acid analyses by gas-liquid chromatography as described previously(1). The remainder of the rat was then saponified in its own weight of 30% ethanolic potassium hydroxide containing 2-3% pyrogallol and an amount of 5-methyl-C^{14}-*d*-α-tocopherol (specific activity 1.1 μC/μmole) comparable to the quantity of α-tocopherol anticipated to

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† Activation Analysis Service, General Atomic Division, General Dynamics, San Diego, Calif.

TABLE I. Urinary Creatine to Creatinine Ratios.

Group No.	1	2	3	4
Dietary fat	Unsaturated*	Unsaturated	Saturated†	Saturated
Tocopherol (mg/kg rat/wk)	0	15	0	0.4
(wk)	Creatine to creatinine ratios‡§			
3	.920 ± .542 (32)	.341 ± .138 (12)		
5	2.23 ± 1.01 (29)	.249 ± .116 (12)		
8			.220 ± .097 (9)	
10			.316 ± .062 (6)	
12			.372 ± .022 (3)	
				.185 ± .069 (80)‖

* Fatty acid composition: saturated 62.2%, monenoic 8.2%, dienoic 5.9%, trienoic 23.7%.
† Coconut oil.
‡ Avg ± S.D.
§ No. of samples in parentheses.
‖ Urine collections at 7, 9, 11, 14 and 16 weeks with average values of creatine to creatinine ratio of .224, .150, .196, .198, and .216, respectively, were combined for statistical purposes. The probability of a urinary creatine to creatinine ratio greater than .368 occurring in the tocopherol supplemented group is .005.

be present in the carcass. Non-saponifiables were extracted into petroleum ether, freed of cholesterol by extraction with 85% (v/v) sulfuric acid, and chromatographed on secondary magnesium phosphate(9). The eluted fraction containing α-tocopherol was analyzed by the method of Emmerie and Engel(10) and by gas-liquid chromatography at 210°C on a 6′ × 3 mm glass column containing Gaschrom-P coated with 3% QF-1, using argon carrier gas and a tritium diode detector. At each stage of the procedure, appropriate aliquots were counted in a scintillation counter and analyzed by the method of Emmerie and Engel(10). On the basis of the added 5-methyl-C^{14}-*d*-α-tocopherol, recoveries were in the range 85-102% at each stage.

Creatine and creatinine were determined on 24-hour urine samples by the method of Bonsnes and Taussky(11).

Results. The rats fed the saturated fat, coconut oil, received marginal quantities of the essential fatty acid, linoleic acid, as evidenced by the occurrence of small quantities (2%) of eicosatrienoic acids in the muscle phospholipids. Muscle neutral lipids contained the same low level of linoleate present in the dietary fat. Rats fed the unsaturated fat had slightly more polyunsaturated fatty acids (PUFA) (48% *vs* 43%) in their muscle phospholipids and had 8 times as much PUFA (23% *vs* 3%) in the neutral lipid fraction of this tissue compared to the coconut oil fed groups. Complete data on muscle lipid fatty acid composition have been presented previously(1) for rats fed these 2 fats.

Both groups (Groups 1 and 3) were depleted of whole body α-tocopherol at the rate of approximately 27 μg/week (Fig. 1a). These data have also been plotted in terms of average whole body level of α-tocopherol, μg/g, wet weight of tissue in Fig. 1b. When the rats were fed unsaturated fat, Group 1, urinary creatine to creatinine ratios were 2.6 and 9 times those seen in the comparable tocopherol supplemented group, Group 2, at 3 and 5 weeks, respectively (Table I). At 3 weeks, 18 of 32 rats had creatine to creatinine ratios significantly ($p = .005$) greater

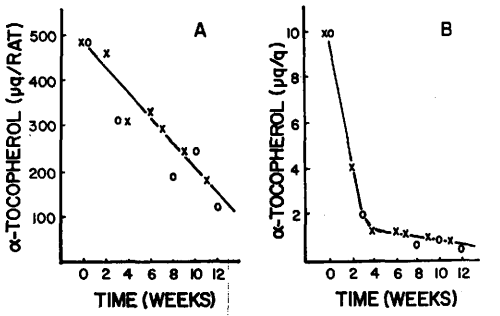

FIG. 1a. Whole body levels of α-tocopherol in rats fed a tocopherol-deficient diet containing either a saturated (O) or unsaturated (X) fat. Each point is the average of 3 animals.
FIG. 1b. Average tissue α-tocopherol levels, μg/g wet weight of tissue. Symbols as above.

than that ratio expected for a tocopherol supplemented rat. The onset of creatinuria has been previously(1) described as the week during which one-half of the group first shows a significantly ($p=0.005$) elevated creatine to creatinine ratio.

While the ratio of urinary creatine to creatinine was elevated in the group fed the saturated fat, Group 3, when compared to the appropriate tocopherol supplemented group, Group 4, these groups of animals did not show a significant creatinuria by the above criterion within 12 weeks. It seems probable, however, that the onset of significant creatinuria was imminent in this group at the end of the experiment.

Discussion. Solely on the basis of the data described here, it might be argued that the tocopherol-deficiency sign, creatinuria, is not related to the depletion of tissue tocopherol stores in the rat. Furthermore, it might even be argued that since the dietary fat did not affect the rate of α-tocopherol depletion, neither dietary nor tissue PUFA affect the tocopherol requirement of the rat.

The data, however, are also completely consistent with the kinetics of lipid peroxidation *in vivo*. Lipid peroxidation proceeds *via* a free-radical initiated chain reaction

$$\left[\begin{array}{l} \rightarrow R\cdot + O_2 \rightarrow RO_2\cdot \\ RO_2\cdot + RH \rightarrow R\cdot + ROOH \end{array}\right]$$

(4,5,12). In the absence of side reactions and free-radical chain terminators, one free-radical initiation could conveivably result in complete peroxidation of all the lipid in a given system; for instance, a mitochondrial membrane. Only one molecule of tocopherol would be destroyed in terminating this free-radical chain reaction $RO_2\cdot + AH \rightarrow ROOH + [A\cdot]$. In practice the number of cycles the chain reaction proceeds is dependent upon tocopherol concentration and on the magnitude of n in $CH_3(CH_2)_a(CH=CH\ CH_2)_n(CH_2)_b\ CO_2H$. Tocopherol is a competitive inhibitor of lipid peroxidation and its relative efficacy decreases markedly (18-fold) as n increases from 1 to 6(1,5).

For rats in the same environment, fed essentially the same diets, differing only in fatty acid composition, exposure to free-radical initiations should be comparable between groups. Some definite fraction of the free-radical chain reactions thus initiated should be terminated by α-tocopherol. It is not, therefore, surprising that both groups showed a similar loss of α-tocopherol over a period of 12 weeks. In the group fed the unsaturated fat there was 8 times as much PUFA in the muscle neutral lipids and somewhat more PUFA in the muscle phospholipids than was found in the corresponding lipids of the group fed the saturated fat. It would be expected that at any given tissue tocopherol level there would be a greater number of cycles of the chain reaction occurring, more lipid hydroperoxide formed per free-radical initiation, prior to termination by the antioxidant, in the tissue of the group fed the unsaturated fat compared to the group fed the saturated fat.

Elsewhere(13,14), it has been shown that in these rats not supplemented with α-tocopherol, fed the unsaturated fat diet, there was a progressive net loss of PUFA from the muscle phospholipids and a concomitant appearance of peroxidized lipid in the form of fluorescent pigment of the lipofuscin or ceroid type; whereas, these phenomena were not seen in the muscles of the rats fed the saturated fat diet. It has also been shown previously(1), however, that a loss of PUFA from the muscle phospholipids of the group fed the saturated fat did occur after a prolonged feeding period, six months to one year(1).

It seems reasonable to relate the onset of creatinuria in the rat to the rate of fatty acid peroxidation in the muscle tissue, which in turn is dependent on the relative efficacy of α-tocopherol as a competitive inhibitor of the peroxidation of fatty acids of various degrees of unsaturation. As noted above, however, other interpretations of the data may be possible.

Summary. Rats fed a diet containing either a saturated or an unsaturated fat were depleted of α-tocopherol (whole body) at comparable rates. Upon depletion to an average tissue α-tocopherol level of 2.4 μg/g wet weight of tissue, the group fed the unsatu-

rated fat showed a significant creatinuria. Depletion of the saturated fat-fed group to an average tissue α-tocopherol level of 0.4 μg/g did not produce creatinuria.

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Species Difference in Hormonal Control of Intestinal Weight and Food Intake of Rats and Pigeons. (30636)

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Bates *et al*(1) have shown in hypophysectomized pigeons that replacement therapy with a combination of four pituitary hormones, growth hormone, prolactin, TSH and ACTH, caused a large body weight gain and food intake, and larger than normal organ to body weight ratios for the intestine, liver, kidney and pancreas. These data indicated a specific effect of the exogenous hormones on the size of these organs. Later studies by Milkovic *et al*(2) in rats with mammotropic tumors, MtT, suggested that the high levels (>30 times normal) of growth hormone, prolactin and ACTH in the blood of these rats did not specifically enlarge the intestine and pancreas, yet they caused large livers, kidneys and hearts.

There is a paucity of data in the literature in which both food intake and intestinal size have been measured. This paper presents a look at certain accumulated data in rats and pigeons where both empty gut weight and food intake were measured.

Methods and materials. The transplantable pituitary tumor, used in these studies, was the mammotropic tumor (MtT), strain F4, which was grown in Fischer rats and was originally obtained from Dr. Furth. We have had it for over 35 transfers. Most of the data here was from our transfers 20 to 30. The tumor had a latent period of growth of about 30 days. Fischer rats about 4 to 6 months old were used throughout. The pigeons were all 6-8 weeks of age of the white Carneau strain.

Most of the rat data is from a study to be published on the induction of diabetes in partially pancreatectomized rats. The percentage of the pancreas removed was checked by weighing the pieces removed during surgery. Partial pancreatectomy and adrenalectomy was usually done after tumor implantation and before the tumor became palpable and began to produce hormones. Severe diabetes, with blood sugars around 300 mg% and daily excretion of 2 to 5 g of glucose in the urine, developed in most rats with MtT if more than 40% of the pancreas was removed and the adrenals were intact.

The intestines, from the pylorus to the anus in rats and from the gizzard to the cloaca in pigeons, were weighed after removing the contents by stripping and/or cutting (especially the cecum).

Except for fasting or force feeding in a