That the reflexes concerned with the turn-over test developed earlier than did those involved in the air-righting response is in accord with the work of Windle and Fish(4), who reported that body-on-body righting reflexes appeared in the fetal cat before labyrinthine reflexes could be observed.

The delayed development of the air-righting response in manganese-deficient offspring may be related to (a) impaired labyrinthine function(5,6,7) (although Hill *et al.*(8) found no differences between normal and manganese-deficient rats in histological studies of the inner ear), and (b) delayed or abnormal myelination. Windle *et al.*(9) observed some correlation between development of labyrinthine reflexes and myelination in the fetal cat. Furthermore, in the normal animals studied in the present experiments, the air-righting response appeared at the age at which myelination is being accomplished(10). The work of Eayrs and Taylor(11) indicates that the thyroid also may be involved, since thyroidectomized animals showed some delay in development of this response. These possibilities are currently under investigation.

Summary. Development of righting reflexes was studied in offspring of manganese-deficient and normal rats by 2 tests: (1) time required to turn from their backs to their feet and (2) ability to right themselves in air when dropped upside down. The reflexes concerned with the turn-over test developed at an earlier age than did those involved in the air-righting response. In both tests, however, the manganese-deficient young exhibited a marked delay in development of reflexes responsible for righting reactions.

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Effect of Vit. E-Deficiency on Protein Composition of Guinea Pig Skeletal Muscle.* (25249)

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Several reports concerning the status of one or more muscle protein fractions from animals maintained on a vit. E-deficient diet have appeared in the literature. Spencer *et al.*(1) observed an increase in collagen content of nutritionally dystrophic rabbit muscle. The myosin and actomyosin content of rabbit skeletal muscle fibers gradually decreases with increasing duration of the deficiency state(2). Baechtel *et al.*(3) noted that nitrogen concentration of water soluble extracts of muscles from supplemented and deficient rabbits were the same. In 21-day old vit. E-deficient rats, Rumery *et al.*(4) found a pronounced loss in actomyosin associated with a marked elevation of insoluble protein. The present investigation was initiated to examine the effect of vit. E-deficiency on the sarcoplasmic, contractile and collagen fractions of skeletal muscles from young guinea pigs. It was also of inter-

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TABLE I. Means and Standard Errors of Total Nitrogen, Sarcoplasmic Nitrogen, Contractile Nitrogen, and Collagen Nitrogen of Gastrocnemius and Masseter Muscles of Guinea Pigs.

Regimen and duration	No. of cases	mg nitrogen/g fresh muscle			
		Total N	Sarcoplasmic N	Contractile N	Collagen N
		Gastrocnemius			
15 days Def.	7	31.18 ± 1.51	7.70 ± .62 (24.7 ± .78)	16.88 ± 1.02 (54.2 ± 1.55)	6.60 ± .21 (21.1 ± 1.89)
" Suppl.	7	31.56 ± 1.91	8.02 ± .67 (25.4 ± .87)	17.25 ± 1.07 (54.6 ± .95)	6.30 ± .11 (20.2 ± 1.30)
21 days Def.	13	31.06 ± .65	8.32 ± .34 (26.8 ± .70)	15.05 ± .56 * (48.5 ± .89)	7.69 ± .32 * (24.8 ± .81)
" Suppl.	10	32.34 ± .93	8.22 ± .28 (25.4 ± .89)	18.07 ± .66 (55.9 ± 1.01)	6.05 ± .35 (18.7 ± .76)
" Recovery	10	33.17 ± .64	8.54 ± .22 (25.7 ± .65)	17.56 ± .46 (52.9 ± .81)	7.07 ± .23 (21.3 ± .33)
30 days Def.	7	31.72 ± .45	8.73 ± .47 (27.4 ± 1.13)	14.87 ± .18 * (47.0 ± .93)	7.97 ± .10 * (25.2 ± .50)
" Suppl.	6	31.88 ± .33	8.31 ± .23 (26.7 ± 1.18)	17.00 ± .78 (53.3 ± .70)	6.56 ± .28 (20.5 ± .76)
		Masseter			
15 days Def.	7	30.81 1.61	8.65 ± .80 (28.1 ± 1.15)	16.00 ± .91 (52.0 ± 1.55)	6.16 ± .71 (20.0 ± .63)
" Suppl.	7	31.59 ± 1.82	8.39 ± .37 (26.5 ± 1.90)	17.26 ± 1.33 (54.8 ± .74)	5.94 ± .47 (18.8 ± 1.57)
21 days Def.	13	32.00 ± .61	8.32 ± .23 (26.1 ± .60)	15.99 ± .46 (50.0 ± .86)	7.69 ± .22 * (24.0 ± .61)
" Suppl.	10	32.85 ± .87	8.68 ± .26 (26.4 ± .57)	18.05 ± .88 (55.0 ± .31)	6.11 ± .35 (18.6 ± 1.24)
" Recovery	10	32.60 ± .59	8.49 ± .22 (26.0 ± .81)	16.94 ± .51 (52.0 ± 1.01)	7.17 ± .26 (22.0 ± .42)
30 days Def.	7	30.84 ± .46	8.22 ± .42 (26.6 ± 1.34)	15.79 ± .28 (51.2 ± 1.05)	6.85 ± .47 (22.1 ± 1.53)
" Suppl.	6	31.44 ± .64	8.59 ± .47 (27.3 ± 1.50)	16.56 ± 1.71 (52.8 ± 1.03)	6.29 ± .51 (19.9 ± 1.60)

Values in parentheses indicate percent of total nitrogen. * Probability $\leq .01$.

est to study the effect on both red (masseter) and pale (gastrocnemius) muscle, for it has been reported(5) that the masseter muscle is the most resistant to nutritionally induced dystrophy.

Methods. Muscle samples employed in this study were obtained from 30-day old male guinea pigs placed on vit. E-deficient and vit. E-supplemented diets for periods of 15, 21, and 30 days. Details of the dietary regimen have been described(6). The method of chemical dissection proposed by Robinson(7) was adapted for use with small samples (15-30 mg fresh muscle), which were removed from central areas of the muscles with the purpose of excluding, to a large measure, extraneous connective tissue. After the fractionation by means of weak and strong salt extracts, nitrogen content was determined by Kjeldahl digestion followed by direct nesslerization. Results of the nitrogen analyses were expressed as concentration (mg/g fresh muscle) and as percentage of total nitrogen; i.e., the sum of sarcoplasmic, contractile, and collagen nitrogen. A statistically significant difference was accepted only when nitrogen concentration as well as percentage of total nitrogen exhibited a probability of ≤ 0.01.

Results. Table I summarizes the results of nitrogen analyses. When compared to their supplemented controls the experimental gastrocnemius muscles in the 21-day study show, in terms of total nitrogen, a decrease in contractile fraction and an increase in collagen fraction of 7.4% and 6.1% respectively. After 30 days on the dietary regimen, the cor-

responding changes are: contractile − 6.3%, collagen + 4.7%. These differences are significant. In contrast, the masseter data reveal a significant alteration that is limited to the 21-day collagen fraction (+ 5.4%). The sarcoplasmic fraction of either muscle exhibited no significant changes at 21 or 30 days. Supplementation after 14 days on the deficiency diet (both control and recovery animals received 15 mg dl-alpha-tocopherol daily from 15th through 20th days) stabilizes the collagen content of both muscles at or close to 15 day deficient levels; and produces a slight, though not significant, increase in the contractile component of the gastrocnemii. The contractile fraction of recovery masseters is equal to that found for 15-day deficient masseters.

Discussion. Values for nitrogen fractions of control muscles expressed as % of total nitrogen agree closely with those obtained by Hasselbach and Schneider(8) using a somewhat similar extraction procedure on normal rabbit skeletal muscle. With increasing durations of the deficiency state in guinea pigs, there are significant alterations in the contractile and collagen components of the gastrocnemius muscles. On the other hand, with the exception of the 21-day collagen data, masseter muscles reveal no significant change attributable to lack of vit. E. Such results were anticipated by the histological studies of Goettsch and Pappenheimer(5).

According to Spencer *et al.*(1) the gastrocnemius collagen nitrogen concentration of rabbits placed on a vit. E-deficient diet at approximately 30 days of age increased slightly after 4 weeks and markedly after 5 weeks (2x). These authors remark that collagen concentration may be augmented even before outward signs of dystrophy become apparent. In young guinea pigs used for the present study there is a significant change in collagen prior to such evidences of dystrophy as elevated urinary creatine, gross cytological lesions, or decrease in muscle weight.

It is the opinion of Bonetti *et al.*(2) that the decline in actomyosin and complete loss of myosin from dystrophic rabbit skeletal muscle is not merely due to a diminished extractability, but to a real loss of such proteins. For comparison it should be noted that their animals were sacrificed after a sharp decline in body weight had occurred.

Total nitrogen extractable with distilled water from skeletal muscle of rabbits showing typical signs of dystrophy is the same as from control animals(3). The results of the present study on guinea pigs tend to confirm the above findings, but distinctly precede them in terms of severity of the dystrophic state.

Finally, it is of interest to note that the alterations in contractile and collagen protein which occur are matched concurrently by changes in myoglobin concentration(6).

Summary. The gastrocnemius muscles of 30-day-old guinea pigs subjected to a vit. E-deficient diet for 21 or 30 days undergo a statistically significant increase in collagen nitrogen and decrease in contractile protein nitrogen. Masseter muscles are more resistant to the effects of vit. E-lack on the protein components. Sarcoplasmic nitrogen is not altered by 21 or 30 day dietary deficiency regimens.

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