TABLE I.

Animal	No.		No. days collected and computed		Pregnandiol Glucuronidate
Rabbits					
Normal mature ♀	2		33		0
Pregnant ♀	2		28		0
Cats					
Normal mature ♀	1		40		0
Pregnant ♀	3		33		0
		Weight in g	Pretreatment with estradiol benzoate	Progesterone injected mg in days	
Adult monkeys					
Castrate ♀	1	4450	0	45 in 10	0
Castrate ♀	1	5700	0.4 mg in 10 d	50 in 10	0
Castrate ♀	1	4750	1.2 mg in 20 d	10 in 10	0
Normal ♂	1	5350	0	30 in 3	0
Normal ♂	1	5000	0	80 in 4	0

No pregnandiol glucuronidate was recovered in any instance. Also after hydrolysis no free pregnandiol was found in the monkey which received 80 mg of progesterone.

Table I shows the type and number of animals used.

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Vitamin E Deficiency in Dogs.*

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In a more extensive experiment designed to compare the nutritive values of raw, pasteurized and evaporated milks we have encountered an acute vitamin E deficiency in dogs. Since a deficiency of vitamin E has not been reported previously in this species we wish to take this opportunity to do so.

Weanling fox-terrier pups were fed a diet of a commercial evaporated milk (neither sweetened nor irradiated) which was diluted

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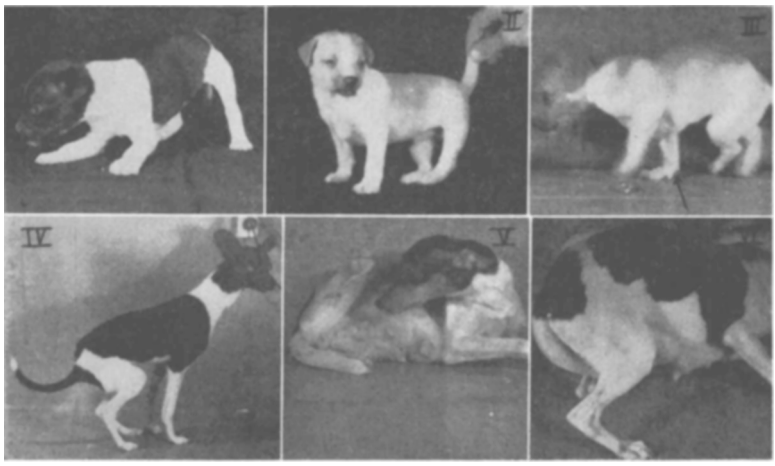
1:1 with water and placed in earthen containers suspended from the side of the pen to prevent waste and contamination. Minerals,† and cod liver oil‡ were added to the morning ration.

No sign of a nutritional deficiency appeared in these dogs except under the added strain of gestation and lactation. After 42 weeks female No. 1 became pregnant and produced a litter of 3 pups, one of which survived only a few hours, the others developed signs of muscle dystrophy about the twentieth day (Figs. I and II).

Faulty bone development was apparently not responsible for the condition since X-ray photographs showed no abnormalities. The greatest weakness appeared in the joint between the femur and tibia but, as shown in Fig. III, the muscles of the fore limb were also affected. A lack of muscle tonus in general and a hyper-sensitivity to pain seemed to be characteristic of the deficient animals. The marked denudation of the head and limbs, together with the dry and feverish skin suggested a cretin-like condition.

Various attempts were made to alleviate these symptoms. Subcutaneous injections of lysine and oral doses of liver extract powder



FIGS. I and IV, Dog No. 6. Note abnormal position of joint between femur and tibia.

FIG. II, Dog No. 7. Note crossing of hind legs as he walks, also flat-footed position of front foot.

FIGS. V and VI, Dog No. 1. Note lesions around teats and on the feet. Note also denudation on dog's head in Fig. V.

† Minerals were added at the rate of 5 mg of iron as the pyrophosphate, 1.0 mg of Cu as the sulfate, and 1.0 mg of Mn as the chloride per dog per day.

‡ Abbott's Cod Liver Oil administered at a level of 1 g per dog per day.
Vitamin D content $= >$ 100 U.S.P. units per g.
Vitamin A content $= >$ 1500 U.S.P. units per g.

had little or no effect, while wheat germ brought about a response in growth and an improvement in the general appearance of the dogs. No. 6 was then supplemented with dl-α-tocopherol and showed a marked improvement in vitality, muscle tonus and growth but continued administration of the crystalline vitamin over a period of 7 months has not cured the deformed posture as is shown by Fig. IV. It appeared then that we were dealing with a vitamin E deficiency but that the condition of muscle dystrophy was too far advanced to be cured.

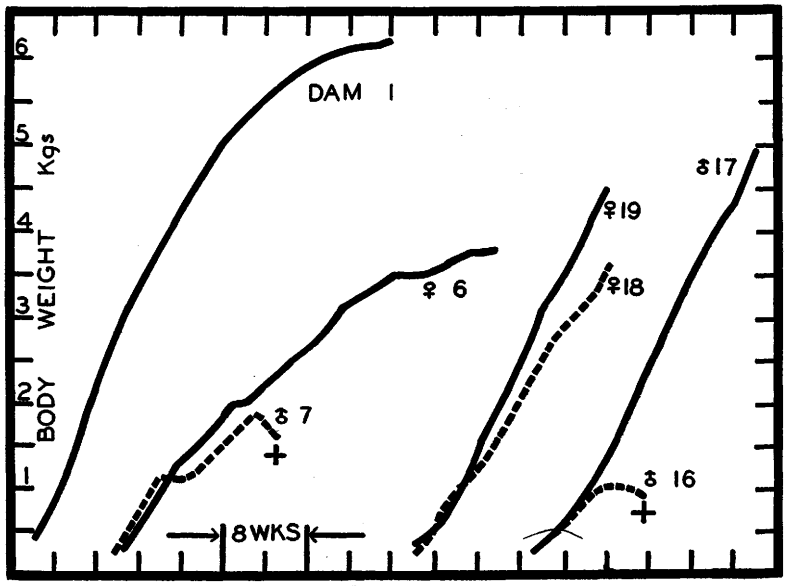
Pups of the second litter of female No. 1 likewise developed the early signs of dystrophy about the twentieth day. At this time, however, the dam also developed signs of a severe deficiency. Besides a marked loss in weight she developed sores on her teats, feet and other areas subjected to abrasion (Figs. V and VI). Her condition was such that it was thought advisable to remove the pups and raise them on the evaporated milk. Two of the pups, No. 17 and No. 19 received 5 mg of dl-α-tocopherol-acetate per week, while No. 16 and No. 18 were maintained as controls. It was apparent that the evaporated milk contained more vitamin E than the dam's milk, since the pups soon overcame the early symptoms of dystrophy.

The 2 pups (No. 17 and No. 19) which received the added vitamin E grew rapidly and, as is shown by the growth curves, these are the only ones which approximate the rapid growth of the original female dog. The pups which did not receive the added vitamin E grew much more slowly and the male pup No. 16 became so emaciated that he was sacrificed for histological examination although distinct muscle dystrophy had not set in.

The fact that in both litters the male dogs developed the deficiency symptoms earlier and to a greater degree indicates that the male dogs are more susceptible to this deficiency than are the females.

The general body appearance was distinctly different in the 2 groups of dogs. Those receiving the vitamin E supplement were sleek, fat pups with firm limbs and an abundance of vitality. Those receiving no vitamin E supplement became emaciated, with thin limbs and a marked weakness in their feet. Instead of standing firmly on their toes they sagged at the joints, often unable to keep their toes from curling (Fig. III).

Histological examination of the tissues showed a degeneration of the thyroid of dog No. 7, but no such changes in the thyroid of dog No. 16. The liver of dog No. 16 appeared to have undergone some hydropic changes and the Hassall bodies of the thyroid were very prominent. These differences may be partially explained by the rela-



GRAPH 1.

Growth curves: Dam 1—one of the original litter. No. 6 and No. 7—pups of dam 1—first litter—weaned by dam—developed muscle dystrophy about twentieth day. Nos. 16, 17, 18, and 19—pups of the second litter—transferred to evaporated milk on twentieth day—showed early signs of dystrophy—apparently recovered when transferred to evaporated milk. Nos. 17 and 19 received 5 mg vitamin E per week.

tive severity of the deficiency symptoms of the 2 dogs. No. 7 had developed the severe muscular paralysis, while No. 16 had shown some of the early signs of this paralysis but had recovered apparently when transferred to the evaporated milk, midway in the suckling period. Further studies on the other tissues have not been completed, but those which have been made, together with the gross symptoms and effects of supplementation point to the fact that this condition of paralysis is identical to the muscle dystrophy due to a vitamin E deficiency reported in other species.

Dam No. 1, when separated from her pups, was supplemented with 10 mg of vitamin E per week since the deficiency symptoms in the pups indicated that vitamin E might be the limiting factor.

Besides the open sores on her body, the skin on her belly appeared pale, wrinkled, cold to touch, and lifeless. There was a general denudation which was most prominent around the head and legs (Figs. V and VI). Eight weeks after the administration of the vitamin E these symptoms had disappeared, only slightly reddened areas remaining to indicate the position of former lesions.

Whether or not the vitamin E was the only limiting factor has not

been determined. Removal of the strain of lactation may have accounted for some of the effects. The answer to this problem will be forthcoming when she has produced another litter with the vitamin E supplied in suitable quantity.

Discussion. It has been clearly demonstrated that pups born to dogs on this ration of commercially evaporated milk will develop a vitamin E deficiency. This deficiency, if allowed to progress, will result in a muscular paralysis.

Nutritional muscle dystrophies have been reported in rats,[1] guinea pigs,[2] and rabbits,[3] on diets low in vitamin E. Attempts to produce similar conditions in dogs and cats,[3] on the diets used for rabbits, have apparently failed. Had these experiments with dogs and cats been continued long enough to include gestation and lactation, it is possible that the young would have developed muscle dystrophy.

Evidence has been produced[4] indicating that suckling rats born to E-low mothers *die*, not from lack of milk, but from a deficiency of vitamin E. If the factor were supplied or if the pups were transferred to normal mothers they survived. The secretion of vitamin E in human milk seems to be governed by the same factors since sterile E-low rats may be cured by feeding human milk,[5] providing the woman had been receiving a diet adequate in vitamin E.

These facts indicate that females conserve vitamin E for their own body needs at the expense of their suckling young. On a diet moderately low in this factor, it is reasonable to assume that the dam's milk would be still lower in this essential factor. Pups raised to weaning on such a milk would quite likely show more severe deficiency symptoms than pups which received the evaporated milk for half of their normal suckling period. Such was the case in this experiment but the change to the evaporated milk did not supply all of the essential factor as is shown by the growth response when dogs No. 17 and No. 19 received additional vitamin E.

Preliminary data indicate that raw milks may also be low enough in vitamin E to allow the development of similar conditions in the second generation pups. Thus the low supply of vitamin E may not be associated exclusively with milks that undergo processing. Since the diet solely of mineralized milk is an abnormal one for an adult dog the limiting amount of vitamin E may not be of great practical

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3 Morgulis, S., *Monographie Actualites Scientifiques et Industrielles*, Hermann and Cie, Paris, 1938, p. 74.

4 Barrie, M. M. O., *Nature*, 1937, **140**, 426.

5 Muller, C., *Schweitz Med. Wschr.*, 1936, **66**, 1164.

significance. The excellent performance of the dogs on mineralized milk until reproduction indicates that milk is adequate in vitamin E during the growing period for which milk is intended.

Summary. A deficiency in pups produced from dogs maintained for long periods of time on mineralized evaporated milk has been described. The condition is undoubtedly identical with muscle dystrophy previously described in rats, guinea pigs and rabbits and is cured by synthetic α-tocopherol if therapy is initiated before the symptoms are too far advanced.

11041 P

Infection of Guinea Pigs by Application of Virus of Lymphocytic Choriomeningitis to Their Normal Skins.

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The virus of lymphocytic choriomeningitis has been found to be infective for animals by a variety of routes[1–4] including, in addition to the more common ones, the intranasal,[2] intravaginal, intraurethral[5] and intrarectal.[6] Furthermore, it has been reported by Findlay and Stern[3] that, when this virus was fed to mice or applied to their lightly scarified skins, the mice did not exhibit apparent infection but the virus could be recovered from their spleens and kidneys. They also showed that when the virus was rubbed on the lightly scarified skins of 2 Rhesus monkeys, one showed a slight febrile reaction, the other no response. Recently, Shaughnessy and Milzer[7] demonstrated that the virus caused typical symptoms of the disease in guinea pigs when placed on their very lightly scarified skins.

The W. E. strain[8] of lymphocytic choriomeningitis virus was employed in these studies. Its virulence was such that, when 0.25 cc

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6 Shaughnessy, H. J., and Zichis, J., unpublished studies.

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