spotted fever obtained through the courtesy of Dr. Dyer of the National Institute of Health.

Cultures were initiated with spleen and tunica by the agar slant method[2, 3] from one of the original guinea pigs. Almost all tubes showed good growth with some intranuclear Rickettsiae by the 10th to 14th day, at which time transfers were made to new slants, with mouse embryo as the fresh tissue. Growth has continued very rich through 10 generations, and transplants may now be made as early as the 6th day. Minced chick embryo may also be used with equally good results.

Guinea pigs inoculated with ½ a slant of the 6th generation mouse culture reacted as shown in Chart 1. Sections of tissues from these animals, examined for us by Dr. Henry Pinkerton, were characteristic of spotted fever. The cultures are still being maintained in series.

10252

Ascorbic Acid Content of Milk of Various Species as Influenced by Ascorbic Acid Injection and Diet.

RUSSEL RASMUSSEN, RALPH BOGART AND L. A. MAYNARD.
From the Laboratory of Animal Nutrition, Cornell University, Ithaca, New York.

This paper reports studies of the ascorbic acid content of the milk of the ewe, cow, mare, and guinea pig. The determinations were made by the titration procedure, using 2,6-dichlorophenolindophenol. The samples were so handled in drawing them from the animal and thereafter as to exclude the light factor which Kon and Watson[1] found responsible for low values. A special microtitration method was devised for studying the guinea pig milk.

A ewe fed a ration of alfalfa hay and grain was milked twice daily for 9 weeks beginning the 12th day after lambing. The yields were recorded and samples were periodically taken for titration. During the last part of the experimental period the influence of ascorbic acid injection was studied. The results are presented in Fig. 1. The data reveal that during the period prior to ascorbic

2 Zinsser, H., Wei, H., and FitzPatrick, F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 285.

3 Zinsser, H., Wei, H., and FitzPatrick, F., *J. Exp. Med.*, in press.

1 Kon, S. K., and Watson, M. B., *Biochem. J.*, 1936, **30**, 2273.

acid injection the values ranged from approximately 25 to 40 mg of ascorbic acid per quart, following a course suggesting an inverse relation to milk yield, as reported for the cow by Rasmussen, *et al.*[2] The intravenous injection of 5 g of ascorbic acid in 20 ml of sterile, distilled water caused a 50% rise in the ascorbic acid content of the milk in 12 hours, followed by a rapid drop to the initial value. A similar result was obtained by a second injection.

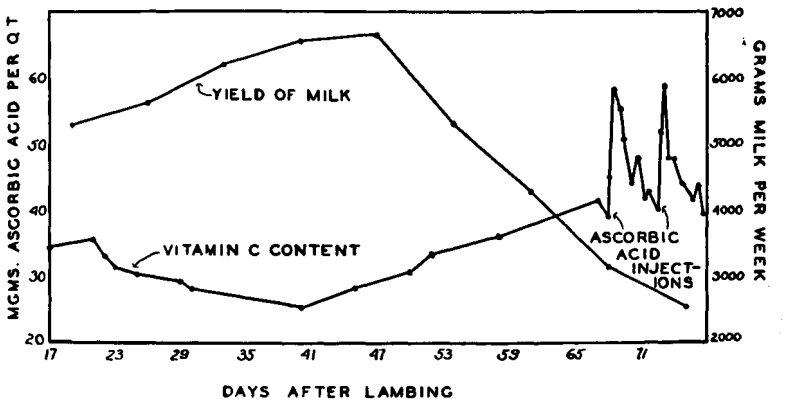

FIG. 1.

The effect of ascorbic acid injection was also studied with two cows, using 24 g in 100 ml of water in one case (Amy) and 12 g in 50 ml in the other (Cleo), with the results shown in Table I.

TABLE I.

	Amy			Cleo		
	Day	Milking	Ascorbic acid per quart, mg	Day	Milking	Ascorbic acid per quart, mg
Before injection	1	8 P.M.	19.2	1	8 P.M.	17.8
	2	8	19.2	2	2	17.8
	3	8	19.2	2	8	17.9
	4	6 A.M.	19.2	3	6 A.M.	17.8
		6:15	Injection		6:15	Injection
After Injection	4	2 P.M.	25.0	3	2 P.M.	22.6
	4	8	30.8	3	8	25.5
	4	12	28.3	4	6 A.M.	22.8
	5	6 A.M.	27.1	4	2 P.M.	19.4
	5	2 P.M.	25.0	4	8	19.4
	6	6 A.M.	21.9	5	6 A.M.	17.2
	6	2 P.M.	22.7	5	2 P.M.	16.4
	6	8	22.7	6	2	16.4
	7	8	20.8	7	2	16.4
	11	2	18.1			
	11	8	18.9			

[2] Rasmussen, Russel, Guerrant, N. B., Shaw, A. O., Welch, R. C., and Bechdel, S. I., *J. Nutrition*, 1936, **11**, 425.

These results support those obtained with the ewe that the injection of ascorbic acid causes an increase in its secretion in the milk. Recognizing that if the injection procedure should disturb the gland in such a way as to increase its permeability to the passage blood constituents any increased titer for ascorbic acid might be meaningless, the milk of the ewe and one cow was analyzed for lactose and chlorides before and after injection. The absence of any significant changes in the concentrations of these constituents indicated that the normal functioning of the gland had not been upset.

The ascorbic acid content of the milk of 6 mares nursing foals was determined beginning at foaling or shortly after and covering a period which included both barn and pasture feeding. A total of 52 samples was examined and the values ranged from 27 to 115 mg per quart. While variations were evident among individuals, the nature of the feed appeared to be an important factor concerned in the wide variations noted for a given mare. The values were markedly higher during periods of succulent pasture than when dried up grass or hay and grain provided the feed. This observation differs from those noted for cows (Riddell, *et al.*[3]). It is felt that definite conclusions regarding the significance of our values obtained with mares should await confirmation by biological studies, since, so far as we are aware, the titration procedure has not been checked with a biological assay in the case of mare milk.

Although we found that small amounts of copper will destroy the titer of mare milk, we also found that sunlight does not decrease this titer to the extent that it does that of cow milk. The latter observation is of interest in connection with the report of Hand, *et al.*,[4] that lactoflavin is the agent in cow milk responsible for the sensitivity of ascorbic acid to light. Our limited studies by the fluorometric method indicated that the lactoflavin content of mare milk is at least very low.

A study was made of the ascorbic acid content of the milk of guinea pigs differently fed as regards the vitamin. The milk of one animal which was receiving a diet of crushed oats, mixed hay, and a limited allowance of green grass gave values of 108, 110, and 112 mg per quart in successive determinations. The milk of a guinea pig receiving all the green grass she would consume gave a value of 397 mg. For another animal receiving the same diet the value was found to be 379 mg. A guinea pig, which received, in addition to green

[3] Riddell, W. H., Whitnah, C. H., Hughes, J. S., and Lienhardt, H. F., *J. Nutrition*, 1936, **11**, 47.

[4] Hand, D. B., Guthrie, E. S., and Sharp, P. F., *Science*, 1938, **87**, 439.

grass, approximately 40 mg of ascorbic acid daily beginning 17 days prior to parturition, secreted milk having a value of 711 mg per quart.

To investigate further the effect of diet upon the ascorbic acid content of the milk of guinea pigs, 5 pregnant animals were placed on a ration designed to be low in the vitamin, a few days before term. All gave birth to apparently normal young, but at the age of 6 days signs of scurvy were evident in the young and this was confirmed by the autopsy of one from each litter. One mother was autopsied also and the typical symptoms found. Two mothers were given 40 mg of ascorbic acid daily at this time while the diet of the other 2 remained unsupplemented. The addition of the vitamin to the diet of the mothers resulted within 6 days in a decrease in the symptoms in the young, while 2 of the young of the mothers which received no supplement died from scurvy on the 10th day, and those remaining alive revealed severe symptoms. The mothers on the unsupplemented diet showed moderate scurvy, while the other showed none. In another experiment 6 pregnant females were placed on the same basal diet, but were removed from their cages daily and allowed to eat all the fresh, green grass they would consume in 2 hours. This procedure was continued after the young were born, but the latter were not given access to the green feed. The young grew normally and showed no signs of scurvy under this regime, in contrast to the marked symptoms which were found in the previous experiment where the mothers were fed throughout on the diet low in the vitamin.

These preliminary studies with the guinea pig provide both chemical and biological evidence that the ascorbic acid content of its milk is influenced by diet. The values, as determined by titration procedure, are surprisingly high, compared to those of the other species studied. They are in accord with the figure of 29 mg per 100 ml reported for guinea pig milk by Houston, Kon and Henry.[5]

Summary. Values for ascorbic acid in ewe milk, determined periodically over the lactation period, ranged from 25 to 40 mg per quart. The intravenous injection of ascorbic acid resulted in a marked, temporary rise in the vitamin content of the milk of the ewe and cow. A study of 52 samples of mare milk gave values ranging from 27 to 115 mg per quart. The nature of the feed appeared to be a factor in the wide variations noted. Values obtained on guinea pig milk ranged from 108 to 711 mg per quart. Both chemical and biological evidence was obtained that the ascorbic acid content of the milk of the guinea pig is influenced by the nature of the diet.

[5] Houston, J., Kon, S. K., and Henry, K. M., *Chem. Ind.*, 1938, **57**, 276.