mental animals prepared by Bouin's fixation and Mallory's stain showed a general though not exact correlation between the completeness of the infundibular lesions and the degree of hyperpigmentation. In the cases of extreme pigmentation the pars intermedia was found to be enormously enlarged showing hyperplasia and cellular hypertrophy, the cytoplasm of the cells showing a marked increase in volume, density and chromophilia. These changes are similar to those shown by successful grafts.

In the light of the previously adduced evidence from grafts, this experiment is interpreted as indicating that the infundibular tracts to the pars intermedia normally inhibit its growth and secretory activity in the tadpole.

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Lactic Acid Formation by Muscles of Scorbutic Guinea Pigs.

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Experiments with washed cells of *Streptococcus lactis* had shown that the rate of conversion of glucose to lactic acid is always increased by addition of peptone, or of nicotinic acid.[1] Ascorbic acid acted irregularly. It proved very efficient only with injured cells, *i. e.*, cells subjected to very long centrifugation, to heating, or to long storage at 0°C. Since the mechanism of acid formation by bacteria is similar to that by animal tissues, the effect of ascorbic acid on the acid formation by muscle of normal and scorbutic guinea pigs was investigated in the hope to find that injury caused by scurvy could be repaired by ascorbic acid.

Preparation of the muscle. Killing of the animal by a blow on the head, followed by skinning and cutting of the muscle which was thrown into solid carbon dioxide, resulted in a very high lactic acid content of the muscle although the entire operation required not more than 10 minutes.

Davenport and Davenport[2] anesthetized the animal, dissected the gastrocnemii free from surrounding tissue, leaving however, blood and nerve connections intact, and after 10 minutes of rest, froze the

1 Rahn, O., and Hegarty, C. P., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 218.
2 Davenport, H. A., and Davenport, H. K., *J. Biol. Chem.*, 1928, **76**, 651.

muscles with solid carbon dioxide. I am obliged to Dr. H. H. Dukes of the Cornell Veterinary College for several such dissections which yielded muscles with very low initial acid content. However, the weight of the muscle was so small, especially with scorbutic animals, that the material was not sufficient for the experiment.

Finally, the entire animal was frozen in carbon dioxide during anesthesia, and all larger muscles of the hind legs were dissected in a cold storage room. The lactic acid content of the muscles was low enough to measure the rate of acid formation.

Conditions of Acid Formation. The frozen muscles were minced with a sharp knife and weighed into test tubes in the cold storage room. For most experiments, 1.0 g was used. All test tubes were placed simultaneously in a water bath, and 1 cc of a solution containing 3% of K_2HPO_4 and 2% glucose[3] was added at once to each tube. For the tests with ascorbic acid, a sufficient amount of this substance had been added to the same solution to deliver 1 mg per cc (100 mg per 100 g muscle). The temperature of the water bath remained constant within 0.1° during each experiment. It was not the same in all experiments because with the advance of summer, the temperature of the tap water rose from week to week.

Analytical Method. The small amounts of muscle made it necessary to use the micro-method of Davenport and Davenport.[2] The

TABLE I.
Lactic Acid Formation in Guinea Pig Muscle (mg per 100 g muscle).

Gastrocnemii obtained by the method of Davenport and Davenport.								
Guinea pig	Normal		Scorbutic II		Scorbutic III		Mixture II + III	
Temp.	20.0°		22.5°		23.3°		21.4°	
Ascorbic acid	0	+	0	+	0	+	0	+
Start	135	—	71	—	—	—	96	104
10 min.	—	—	—	—	148	(18)	151	196
15 "	425	442	—	—	—	—	—	—
20 "	—	—	313	317	151	145	191	207
30 "	—	—	—	—	—	—	262	212

Hind leg muscles obtained by direct freezing.								
Guinea pig	Normal		Scorbutic IV		Scorbutic V		Avg IV + V	
Temp.	25°		25°		25°		25°	
Ascorbic acid	0	+	0	+	0	+	0	+
Start	38	—	59	—	41	—	50	—
10 min.	425	445	188	215	189	222	189	219
20 "	403	517	216	165	238	212	227	189
40 "	573	521	234	210	218	264	226	237
Grand avg	467	494	213	196	215	233	214	215

[3] Meyerhof, O., *Pflüger's Archiv.*, 1921, **188**, 121.

only deviation was an increase in the length of the adsorption tube, and the cooling of the adsorption vessel on hot summer days.

Production of Scurvy. For the feeding and care of the animals, I am much obliged to Mr. Tung Shen, of the Cornell Animal Husbandry Department. The scorbutic animals were killed when the symptoms became so severe that they might not survive another night.

Results. In both sets of experiments given in Table I, the normal muscle produced about twice as much acid as the scorbutic muscle. At 20 to 25°, the maximal amount of acid was reached within 10 to 15 minutes

The second set shows a greater fluctuation of results, because of the greater heterogeneity of the muscle material. While the first set consisted of gastrocnemii only, the second set was made up of several muscles, and contained parts of sinews, fat and connective tissue which might not have been distributed quite evenly in the small samples. The 2 scorbutic guinea pigs have nearly the same rate of acid formation, and the averages show that the differences are not significant.

The injury of the enzyme complex produced by scurvy is not repaired by simple addition of ascorbic acid to the minced muscle. Neither the normal nor the scorbutic muscle showed any significant effect of the added ascorbic acid.

Ratsimamanga[4] had observed that in living guinea pigs, increasing doses of ascorbic acid result in decreasing amounts of lactic acid in muscle and blood, but in increasing amounts of glycogen in muscle and liver. The effect of ascorbic acid on injured cells of *Streptococcus lactis* which had caused this investigation is apparently not a direct effect upon the enzyme complex, but upon the repair mechanism of the cell. It suggests a relation to the observation of Tafel and Harvey[5] that wounds of scorbutic or partly scorbutic guinea pigs heal less rapidly than those of normal animals.

Summary. The excised muscle of scorbutic guinea pigs suspended in an equal volume of phosphate buffer plus glucose, produces only half as much lactic acid as the muscle of normal animals. Addition of 100 mg ascorbic acid to 100 g of muscle had no effect upon the rate or the final amount of lactic acid produced, neither with normal nor with scorbutic muscle.

[4] Ratsimamanga, A. R., *Compt. Rend. Soc. Biol.*, 1937, **126**, 1134.

[5] Tafel, M., and Harvey, S. C., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 518.