

$$q_{n1} = (\varphi_2, \rho_{+1}^2)df_{+1}^2 \pm (\varphi_{-2}, \rho_{-1}^2)df_{-1}^2,$$

$$r_{n1} = \varphi_2 df_{+1}^2 \pm \varphi_{-2} df_{-1}^2, s = df_{+1} df_{-1}.$$

5. A paper giving the above and other developments in particular is to appear in the September (1921) number of the *Annals of Mathematics*. An article relating to the rôle of invariant elements<sup>6</sup> in algebraic orthogonal and boolean invariant theory appeared in the *Transactions* of the American Mathematical Society, vol. 20 (1919), and a paper containing like theory for invariants of relativity and modular invariants appeared in the same *Transactions* for the year 1920. Further researches are in progress.

<sup>1</sup> Elliott, *Proc. London Math. Soc.*, 33 (1901).


<sup>2</sup> Christoffel, *Crelle, J. Math.*, 70 (1869).

<sup>3</sup> Ricci and Levi-Civita, *Math. Ann.*, 54 (1901).

<sup>4</sup> J. E. Wright, *Invariants of Quadratic Differential Forms*, Cambridge tracts (1908).

<sup>5</sup> Maschke, *Trans. Amer. Math. Soc.*, 1 (1900); 4 (1903).

<sup>6</sup> O. E. Glenn, *Ann. Math.*, 20 (1918).

THE EFFECT OF TEMPERATURE AND OF THE CONCENTRATION OF HYDROGEN IONS UPON THE RATE OF DESTRUCTION OF ANTISCORBUTIC VITAMIN (VITAMIN C) 

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In these experiments the vitamin was determined by means of feeding experiments with guineapigs according to the general method made familiar by Hess' recent monograph.<sup>1</sup> Cohen and Mendel,<sup>2</sup> Givens and McCluggage,<sup>3</sup> and the workers at the Lister Institute<sup>4</sup> among other investigators of the antiscorbutic vitamin, have emphasized the importance of basal diets which shall supply all other nutritive requirements and yet furnish none of the vitamin in question or only negligible traces of it. Further investigation of this point led the present writers to adopt the following as an improvement upon the diets previously proposed.

*Basal Ration Used.*—Ground whole oats were mixed with skimmed milk powder which had been heated sufficiently to ensure destruction of such antiscorbutic vitamin as it might contain without so changing the flavor as to cause it to be refused by the experimental animals. The heat treatment necessary to ensure complete destruction of vitamin C in the skimmed milk powder should be determined by each investigator for his own material and technique of heating. In our experiments two hours' heating

at 110° C. in shallow trays freely exposed to air was found sufficient. To the mixture of oats and skimmed milk powder were added pure fresh butterfat and sodium chloride. The proportions adopted were:

Ground whole oats .....	59 per cent
Heated skimmed milk powder.....	30 per cent
Butterfat.....	10 per cent
Sodium chloride.....	1 per cent

By using heated skimmed milk and fresh butterfat instead of heated whole milk, the absence of antiscorbutic vitamin is equally well ensured and the palatability of the diet and its fat-soluble vitamin content are improved. This diet supports excellent growth up to the time of the onset of scurvy.

*Experimental Animals.*—Our experience with about 200 experimental animals indicates that young guineapigs from six to eight weeks old and weighing 300 to 350 grams are best used for studies of vitamin C. Placed upon the above ration at this age and size they usually eat about 18 to 20 grams per day and continue to grow for about 15 days, then lose weight rapidly and die of scurvy in from 26 to 34 days after being confined to the diet. If much older, the animals are somewhat less susceptible; if much younger, the results are less regular.

*Symptoms, Survival Period, and Autopsy Findings.*—The first symptoms appear after about 12 days on the basal diet. In our cases on the above basal diet only, the survival periods were, respectively, 33, 26, 27, 28, 34, 28, 27, 34, 26, 29, 32, 32, 32, 31, 34 days. The nature of the symptoms and autopsy findings agreed so closely with the descriptions of Cohen and Mendel and of Hess as not to require further discussion here. At autopsy the findings which proved most significant were: looseness of teeth, fragility of bones, enlargements and hemorrhages of joints and of rib junctions. When the animal receives some antiscorbutic vitamin but not enough for protection from scurvy, life is prolonged, but to a rather uncertain extent, and some of the symptoms and autopsy findings may become even more severe than in the animals that receive no antiscorbutic and die more quickly.

*Quantitative Rating.*—From what has just been said it necessarily follows that exclusive dependence upon either the survival period or any one set of symptoms or autopsy findings might easily prove misleading. All of these are, therefore, considered in each case. This was done with several series of animals, some receiving the basal ration only, and others measured amounts of filtered canned tomato juice up to the amount which gave complete protection from scurvy. By comparison with the standard protocols thus established, for data of which reference must be made to the fuller account of the work to be published elsewhere, it becomes possible to form a quantitative estimate of the degree of protection (if any)

which the diet, alone or with such antiscorbutic addenda as have been introduced, has afforded the animal.

*Effect of Heating in Acid Solution.*—In the case of tomato juice of natural acidity, pH = 4.3, it was found that boiling for one hour destroyed practically 50%, and for four hours destroyed practically 68% of the antiscorbutic vitamin present. The time curve of the destructive process is, therefore, much flatter than that of a unimolecular reaction or of a reaction proceeding according to the square root rule of Schütz. Similar flattening of the time curves of the destruction of the vitamin were found also in experiments in which the tomato juice was heated at temperatures lower than boiling, viz., at 60° and at 80°. Comparisons of the data obtained at 60°, 80° and 100° show relatively low temperature coefficients:  $Q_{10}$  (60°–80°) = 1.23;  $Q_{10}$  (80°–100°) = 1.12.

*Effect of Reduced Concentration of Hydrogen Ions.*—In experiments in which the natural acidity of the standard tomato juice was first neutralized in whole or in part, the juice then boiled for one hour and immediately cooled and reacidified, it was found that at pH 5.1 to 4.9 (natural acidity less than half neutralized) the destruction during one hour's boiling was increased to 58%. Neutralization of a larger proportion of the natural acidity increased the rate of destruction of the vitamin. When alkali was added to an initial pH of 11, which fell to about 9 during the hour of heating, the destruction found by feeding of the juice thus treated but immediately cooled and reacidified, was about 65%.

In all of these experiments the heating was performed in cotton-stoppered, narrow-necked flasks from which air was probably very largely displaced by water vapor early in the heating. When the experiments upon heating at 100° were repeated with oxygen bubbling through the solution the destruction of the vitamin was much more rapid.

Heating at 100° for one hour at pH = 11 to 9 as described above followed by standing for one to five days in stoppered but only partially filled bottles in a refrigerator at 10° C. at an alkalinity of only pH = 9 was found to destroy 90 to 95% of the antiscorbutic vitamin, as compared with 65 per cent when the solution was re-acidified after heating. This confirms the observations of Harden and of Hess upon the susceptibility of this vitamin to alkalinity even at low temperatures.

Thus while the great instability of the antiscorbutic vitamin makes it an unpromising material for attempts at actual isolation, the development of methods for its quantitative measurement makes possible the study of its chemical behavior.

<sup>1</sup> Hess, A. F., *Scurvy Past and Present*, Philadelphia, 1920.

<sup>2</sup> Cohen, B. and Mendel, L. B., *J. Biol. Chem.*, **35**, 1918 (425).

<sup>3</sup> Givens, M. A. and McCluggage, H. B., *Ibid.*, **37**, 1919 (255).

<sup>4</sup> Chick, H., Hume, E. M., Delf, E. M. and others; A series of papers in *Biochem. J.*, 1918, et seq.