restricted feed intake were similar to those of animals fed cobalt.

These data show that in cobalt deficiency marked alterations occur in the types and numbers of bacteria in ruminants and that these bacteriological changes are not caused by lowered feed intake of the deficient animals.

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Spectrophotometric Determination of Amino Acids by the Ninhydrin Reaction

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In view of the growing interest in the separation of amino acids by partition paper chromatography (*1, 2, 3, 4, 7, 8*), studies have been made to determine if a quantitative relationship might be established in the colorimetric reaction between ninhydrin and the amino acids. Harding and MacLean (*5*) first developed this reaction and later (*6*) condemned it as a colorimetric method for amino acid determination. In mixtures of amino acids they found a lack of specificity and a variation in the red and blue colors produced in the reaction, since ammonia and amines other than amino acids formed similar colors with ninhydrin.

Solutions of 13 amino acids were prepared by dissolving 2 mg of each in 80% ethanol. The ninhydrin reagent was prepared by dissolving 200 mg of ninhydrin in 100 ml of isobutanol. Fifty γ of each amino acid were placed into a test tube, 2 ml of ninhydrin reagent added, and the total volume made up to 10 ml with isobutanol. It was observed in many trials that the color would develop by itself at room temperature. However, to make conditions uniform, all tubes were incubated at 80° C for 3 min, removed, and cooled under running water to 22° C for 3 min. Each tube was then immediately placed in a Coleman No. 6 spectrophotometer, and the transmission determined at 10 mμ intervals between 400 and 700 mμ against a standard containing 2 ml of ninhydrin reagent and 8 ml of isobutanol until the inflection point was approached when the measurements were made at 5 mμ intervals.

The wavelengths corresponding to the inflection points in the wavelength-% transmission curves of amino acids are presented in Table 1.

TABLE 1

WAVELENGTH OF MAXIMUM ABSORPTION OF AMINO ACIDS REACTED WITH NINHYDRIN

Amino acid	Wavelength in mμ	Amino acid	Wavelength in mμ
Phenylalanine	530	Glycine	555
Lysine	545	Methionine	560
Threonine	550	Valine	560
Tryptophane	550	Arginine	560
Alanine	550	Norvaline	560
Asparagine	550	Isoleucine	565
Leucine	555		

Serial dilutions of the amino acids were made and, after reaction with ninhydrin, measured spectrophotometrically at the appropriate wavelength. The quantitative limits in γ, within which it appears possible to measure spectrophotometrically amino acids which have reacted with ninhydrin under the described conditions, that is, the limits at which the points of a plot of concentration vs. logarithm of transmission fall on a straight line, are presented in Table 2.

TABLE 2

LIMITS OF THE SPECTROPHOTOMETRIC DETERMINATION OF AMINO ACIDS REACTED WITH NINHYDRIN*

Amino acid	Concentration (γ per 100 ml)	Amino acid	Concentration (γ per 100 ml)
Phenylalanine	10–140	Threonine	20–130
Isoleucine	20–125	Tryptophane	20–200
Leucine	10–100	Glycine	10– 80
Lysine	5– 50	Alanine	10– 80
Methionine	10–100	Asparagine	20–180
Valine	10–100	Norvaline	10– 70
Arginine	20–100		

* Within these limits the transmission was a straight line.

From the results of these determinations it appears feasible to adapt these studies to the quantitative estimation of amino acids separated by the partition paper chromatographic method. Transmission curves could be determined, and the quantities of specific amino acid present thereby measured in appropriate dilution.

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