

0.5 mgm. per cent may already signify saturation, while in contradistinction 0.9 mgm. per cent may, in certain circumstances, indicate hypovitaminosis.

It seemed probable that intravenous injection of 300 mgm. of ascorbic acid after a previous determination of the vitamin C concentration prevailing in blood may disclose, after repeated determination, whether saturation or hypovitaminosis was originally present. In the latter case, the amount of vitamin injected may be expected to make up the amount and the subsequent determination would disclose this fact by giving the same or an only slightly increased value compared with that of the first determination. If, on the other hand, saturation originally prevailed, the values of the second determination would disclose an increased amount, the organs storing the substance either slowly or not at all.

The validity of this assumption was proved experimentally. If the first determination disclosed less than 1 mgm. per cent concentration, and 300 mgm. were injected, at least double the amount was determined two hours afterwards, indicating that originally saturation dominated. If hypovitaminosis was originally prevalent, the ultimately determined amount was the same as the original, or slightly increased, but never twice as much.

In cases which displayed a concentration higher than 1 mgm. per cent from the start, this was not always doubled after the subsequent injection, the surplus being somehow ejected. In some cases it was possible to ascertain that the ejection was due to the renal function. The practicability of the test is not impaired by this since concentration above 1 mgm. per cent signifies saturation anyhow. All cases were controlled by the Tillmann-Harris-Ray test and the intracutaneous method of Rotter.

For determining the blood concentration, the method of Berend-Fischer was applied, this being sufficiently simple for clinical purposes. Out of forty cases investigated, only one proved a failure.

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#### Free and Bound Vitamin B<sub>1</sub> in Milk

WE have shown<sup>1</sup> that the fluorimetric assay<sup>2</sup> of vitamin B<sub>1</sub> in milk gives values about 50 per cent lower than the biological test. The difference was not due to the presence in milk of free cocarboxylase or of the monophosphate, as in no case could more than a trace of fluorescence due to oxidized phosphorylated aneurin be observed in the aqueous layer after extraction with iso-butanol.

We now find that after incubation of raw milk or of reconstituted dried milk with taka-diastase (containing taka-phosphatase, protease and doubtless other enzymes) at pH 3.7-4.0, the fluorimetric assay is almost doubled. Peptic digestion gives very similar results even when the phosphatases of milk have been inactivated by heating.

It seems probable, therefore, that, in addition to free vitamin B<sub>1</sub> there is present in milk a vitamin B<sub>1</sub>-protein complex. Ultra-filtration experiments support this view. After filtration of milk through 'Cellophane'<sup>3</sup> the serum contains only free aneurin and the substance which yields vitamin B<sub>1</sub> after incubation with pepsin or treatment with taka-

diastase is concentrated in the protein fraction. The recovery is nearly quantitative.

The evidence suggests that the bound vitamin B<sub>1</sub> is not adsorbed but is a constituent of the protein molecule.

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<sup>1</sup> Henry, Houston and Kon, *Chem. and Ind.*, 57, 974 (1933).

<sup>2</sup> Jansen, *Rec. Trav. chim.*, 55, 1046 (1936).

<sup>3</sup> Folley and Mattick, *Biochem. J.*, 27, 1113 (1933).

#### Production of Diacetyl by Faecal Streptococci

IN an investigation of the metabolism of both 'resting' and growing cells of lactic acid bacteria we have found that only the faecal streptococci (*Str. faecalis* and *Str. liquefaciens*) produce considerable quantities of diacetyl, which is recognized to be responsible for part of the aroma of milk products. No other lactic acid bacterium studied in this way produced diacetyl.

The bacteria in massive suspension were held in phosphate buffer plus glucose at pH 6.25, or grown in milk and yeast milk. Diacetyl was only produced in the presence of oxygen. Distillation with ferric chloride greatly increased the yield of diacetyl so that of the acetoin produced in the fermentation only a small part had been oxidized to diacetyl, which was identified by smell, the Vosges-Proskauer reaction and the formation of nickel dimethylglyoxime.

Aroma in butter and other milk products is usually attributed to the fermentation of citric acid by the citric fermenting streptococci<sup>1</sup> and it has also been claimed that the heterofermentative lactobacilli can produce the aroma substances<sup>2</sup>. It may be emphasized that both these groups are quite unable to form the aroma compounds from glucose under 'metabolic' conditions.

#### PRODUCTION OF AROMA CONSTITUENTS UNDER AEROBIC CONDITIONS.

	"Metabolic" tests (Washed cells in glucose-phosphate)		Cultural tests (Growing bacteria in milk and yeast milk)	
	<i>Str. faecalis</i>	Heterofermentative streptococci and lactobacilli	<i>Str. faecalis</i>	Heterofermentative streptococci and lactobacilli
Volatile acid	+	+	0	+
Alcohol	0	0	0	+
Diacetyl + acetoin	+	0	+	0

It is well recognized that milk produced in a very clean manner yields a mild flavoured cheese and that the best and fullest flavoured cheese are frequently made under conditions favouring the seeding of the milk with many organisms, of which the faecal streptococci may be directly concerned in the production of flavour.

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<sup>1</sup> Hammeri, *Res. Bull. Ia. Agr. Exp. Sta.*, 63 (1920).

<sup>2</sup> Bitter, *Landw. Jahrb. Schweiz.*, 218 (1934).