djencolic acid was insoluble. The amino acid was recrystallized three times from 6 N HCl in the form of needles. They were compared with djencolic acid synthesised according to du Vigneaud and Patterson [9]. They had the same R_F-values in paper chromatograms run with different solvents. The X-ray powder diagrams were the same. The molecular weight was 325 as calculated from X-ray diffraction data, suggesting the presence of four molecules per unit cell. The substance had the following composition.

3.855 mg	substance:	0.306 ml N_2 at 28° and 763 mm.
3.813 »	»	3.274 mg CO_2 and 1.539 mg H_2O.
6.464 »	»	32.36 ml 0.01 N thiosulfate (Kirsten)
5.680 »	»	3.584 ml 0.01 N $AgNO_3$

$C_7H_{14}O_4N_2S_2 \cdot 2$ HCl

	C	H	S	Cl	N
Calc.	C 25.6	H 4.9	S 19.6	Cl 22.3	N 8.6
Found	» 23.4	» 4.5	» 20.2	» 23.0	» 9.0

Scarcity of material allowed only a single analysis. The results seem to indicate that djencolic acid is present in the plasma peptide fraction.

A spot corresponding to the position of djencolic acid has also been observed in chromatograms from acid-hydrolyzed plasma, liver and muscle tissues. With regard to the quantitative aspects the chromatograms showed the following pictures when amounts of material corresponding to 100 μg of material were used. The strongest spot was obtained from the peptide fraction. Liver and plasma contained smaller amounts of the amino acid and in muscle hydrolysates the spot was barely visible. Negative results were obtained with hydrolysates of proteins from the spleen and with a peptide fraction prepared from human urine. On the other hand, recent results in this laboratory with chromatograms of some mucoproteins seem to show the presence of a spot close to the position of djencolic acid [10].

Accordingly, our results are in good agreement with the opinion expressed already in 1936 by du Vigneaud [9]: "It is possible that the compound may be more widely distributed in nature and perhaps may be responsible for a portion of the non-cystine, non-methionine sulfur in certain protein." A full account of this work will appear elsewhere.

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Separation of Growth Factors for *Lactobacillus lactis* Dorner *Lactobacillus leichmannii* and *Leuconostoc citrovorum*, by Means of Ionophoresis on Paper

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The usefulness of microorganisms for identifying and estimating biologically active substances is in some cases limited, because of the lack of specificity. In the

determination of vitamin B_{12} by lactobacilli, seven naturally-occurring entities are known to interfere, *viz.* five desoxyribosides and at least two different forms of the vitamin. Little is known about the factors causing growth of *Leuconostoc citrovorum* but, within the range of concentration used for the estimation of natural and synthetic citrovorum factor on agar plates, at least four factors stimulate the growth of this microorganism [1,2]. The introduction of partition chromatography on paper [3] greatly facilitated the separation of the small quantities of substances active in microbiological tests. The first report of a paper chromatographic separation of microbiological growth factors, and the determination of the position of the different factors by using a microbiological indicator was given by Winsten and Eigen [4] on the chromatography of vitamin B_{12} and related growth factors.

In 1948 Wieland and Fischer [5] and later Durrum [6] described a procedure for the separation of amino acids and proteins by electrophoresis or ionophoresis on paper. This method has proved very useful in the study of proteins as shown by Tiselius *et al.*[7] The present paper deals with an attempt to apply paper ionophoresis to the separation of some growth factors for *Lactobacillus lactis* Dorner (ATCC 8000), *Lactobacillus leichmannii* 313 (ATCC 7830) and *Leuconostoc citrovorum* (ATCC 8081). The apparatur used was essentially of the same type as that described by Kunkel and Tiselius [7]. Carbon rods or platinum sheets were used as electrodes. Voltages ranging from 200 to 1 000 volts were applied, the most suitable for our purpose seemed to be 600 volts. The paper strips — 2.5—3 cm broad and about 32 cm long — were cut from sheets of Whatman No. 1 paper. Phosphate buffers (0.03 *M*) which have the advantage of covering a very wide pH range were employed. The ionophoresis experiments were run at room temperature for 1—5 hours depending on the factors to be separated. The paper strips were then dried and placed on agar plates seeded with the microorganism in question.

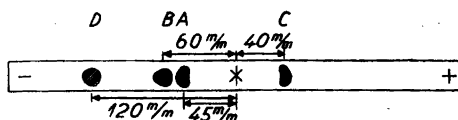

Fig. 1. Separation of vitamin B_{12}-factors by means of ionophoresis on paper. 600 volts, pH 5.0, 4 hours.
Cobemin protected from light: spot A.
Illuminated Cobemin: spots A, B, and C.
Normocytin, Reticulogen and fermentation liquor from Streptomyces griseus: spots A, B, C, and D.
Bacto Liver and Heptomin: A, B, and C.

Vitamin B_{12} or cyanocobalamin (Cobemin, Merck) protected from light migrates towards the cathode both at pH 5.0 and at pH 11.0 (Fig. 1 spot A). When the cyanocobalamin was illuminated two new spots appeared: one caused by a factor moving towards the anode (C) and the other (B) by a factor moving towards the cathod as does A, but faster than A. Normocytin (Lederle) that is supposed to contain only vitamin B_{12} and vitamin B_{12b} (hydroxy- or aquacobalamin) gave rise to four growth zones after ionophoretic separation. Three of these seemed to be identical with the spots A, B and C obtained with illuminated Cobemin, the fourth factor, causing the spot D in figure 1, migrated considerably faster towards the cathode than A and B. All four spots (A, B, C and D) could also be obtained with a fermentation liquor (*Streptomyces griseus*) and with Reticulogen (Lilly), whereas an aqueous extract of Bacto liver and an injectable liver concentrate Heptomin (Tika) only gave rise to three spots (A, B and C).

The spot C might be caused by a complex between the di- or trivalent ions of the buffer solution and the cobalamin, which could explain why it migrates in the opposite direction of B_{12} and B_{12b}. The

factor D, on the other hand, can hardly have been formed from B_{12} or B_{12b} during the ionophoresis as in that case it should appear when illuminated cyanocobalamin is employed. Normocytin, Reticulogen and the fermentation liquor of *Streptomyces griseus* thus contain a third B_{12} factor. It was observed that this factor was destroyed in alkali under conditions which destroy vitamin B_{12}. The factor D seemed to be equally active towards *Lb. lactis* Dorner and *Lb. leichmannii*. It will, however, need further research to demonstrate the nature of this new form of vitamin B_{12} and to decide if it is identical with any of the vitamin B_{12}-factors — different from B_{12} and B_{12b} — reported by other authors [8–10].

To obtain separation of the desoxyribosides (thymidine, desoxyguanosine and desoxyhypoxanthosine were employed) from the B_{12}-factors a pH of about 11 had to be employed. The separation was then complete within 3 hours and it could be shown that very little destruction of vitamin B_{12} had occurred in spite of the alkaline conditions. The desoxyribosides moved towards the anode at this pH, desoxyhypoxanthosine being the fastest moving of the three.

For separation experiments with *L. citrovorum*, only thymidine, synthetic citrovorum factor (Leucovorin, Lederle) and natural citrovorum factor (isolated from horse liver) [11] were available in pure form. Thymidine could easily be separated from the natural or the synthetic citrovorum factor at pH 8.0. At this pH thymidine migrates towards the cathode and both the natural and the synthetic citrovorum factor towards the anode. The separation was complete within 1—2 hours. The natural and the synthetic citrovorum factors seemed to migrate at the same speed towards the anode. The acidic property of the natural citrovorum factor was also observed by Lyman and Prescott [12] in their study on the electrolysis of growth factors for *L. citrovorum*. Thymidine and citrovorum factor occurring in liver extracts, fermentation liquors and in Reticulogen (Lilly) could also be separated by means of ionophoresis on paper.

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