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THE INSTABILITY OF PYRUVATE-C¹⁴

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THE INSTABILITY OF PYRUVATE-C¹⁴

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The Instability of Pyruvate-C¹⁴

In a recent publication von Korff (1) reported on the frequent presence of impurities in pyruvate-C¹⁴ purchased from commercial sources, as well as the decomposition of this oxoacid on storage. He used one-dimensional chromatography, either on paper or on Dowex 1, for the detection of impurities. The situation is indeed worse than that described by von Korff, and this becomes only too clear if one uses for analysis the greater resolving power of two-dimensional paper chromatography.

Sodium pyruvate-3-C¹⁴ (3.62 $\mu\text{C}/\mu\text{mole}$) was purchased from Nuclear Chicago Corp. It was stored unopened for about 2 years at -20° and then dissolved in water at a concentration of 1 $\mu\text{C}/\mu\text{l}$. A sample of the sodium pyruvate solution (containing about 1 μC), together with about 5 μl of buffer, pH 7.4, was spotted onto Whatman No. 4 filter paper and chromatographed using "semi-stench" solvent (2) in the first dimension and butan-1-ol:propionic acid:water (3) in the second. Fig. 1 shows a radioautogram of the developed chromatogram; although much of the C¹⁴ on the chromatogram was still in pyruvic acid, a very large proportion was present in a wide variety of other substances. The area of the chromatogram containing pyruvic acid-C¹⁴ was next eluted with water and the eluted material immediately rechromatographed in "semi-stench" followed by butan-1-ol:ethan-1-ol:water (14:2:5) (Fig. 2). Here, too, several contaminants were observed having chromatographic mobilities in the "semi-stench" solvent very different from pyruvic acid, indicating that they were true decomposition products of pyruvate. Nearly 50% of the C¹⁴ was no longer in pyruvic acid.

It was then decided to prepare pyruvic acid-C¹⁴ in the laboratory in the hope that if it were used sufficiently soon after preparation serious decomposition might be avoided. Pyruvate-U-C¹⁴ was prepared from L-alanine-U-C¹⁴ (3.65 $\mu\text{C}/\mu\text{mole}$) by transamination with heart muscle glutamic-pyruvic transaminase (Boehringer), using sodium α -oxoglutarate as amino acceptor (4); the alanine contained only a trace of radiochemical contamination. The reaction was performed in a small volume and the pyruvate-U-C¹⁴ separated from residual alanine-U-C¹⁴ by chromatography in one dimension on Whatman No. 4 paper in "semi-stench" solvent (Fig. 3). This purified pyruvate-U-C¹⁴ was again chromatographed with "semi-stench" solvent followed by butan-1-ol:propionic acid:water, this time on Ederol No. 202 paper. Once more there was massive breakdown of the pyruvate (Fig. 4), and we have therefore abandoned further attempts to use this substance as a metabolic substrate.

These observations fully support and extend those made by von Korff (1). It is unfortunate that a material of such central metabolic importance responds so unfavorably to simple chemical manipulation. Von Korff reports that storage as the free acid rather than as the sodium salt is more satisfactory. In our case this would not have helped since the pyruvate was analyzed chromatographically immediately after elution from the chromatogram on which it was isolated from the preparative reaction mixture.

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Captions for Figures.

- Fig. 1. Radiochromatogram of commercial sodium pyruvate-3-C¹⁴ after dry storage for two years at -20°.
- Fig. 2. Pyruvate-3-C¹⁴ eluted from the position shown in Fig. 1, and rechromatographed without delay.
- Fig. 3. Isolation of pyruvate-U-C¹⁴ produced by transamination from L-alanine-U-C¹⁴.
- Fig. 4. Pyruvate-U-C¹⁴ eluted from the chromatogram shown in Fig. 3, and rechromatographed without delay.

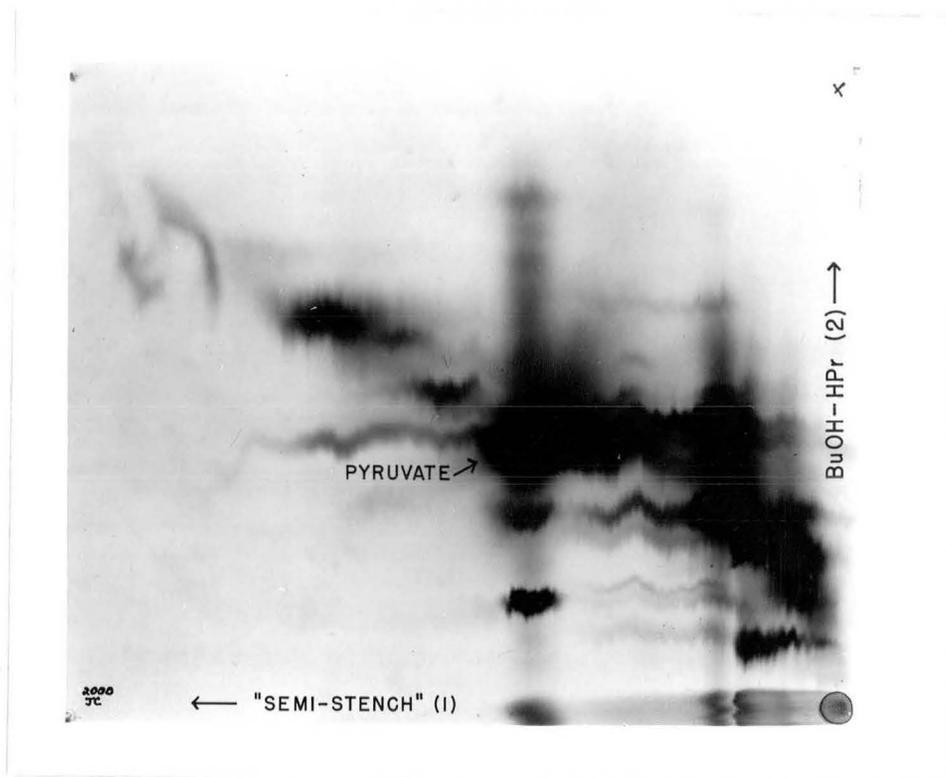


Fig. 1

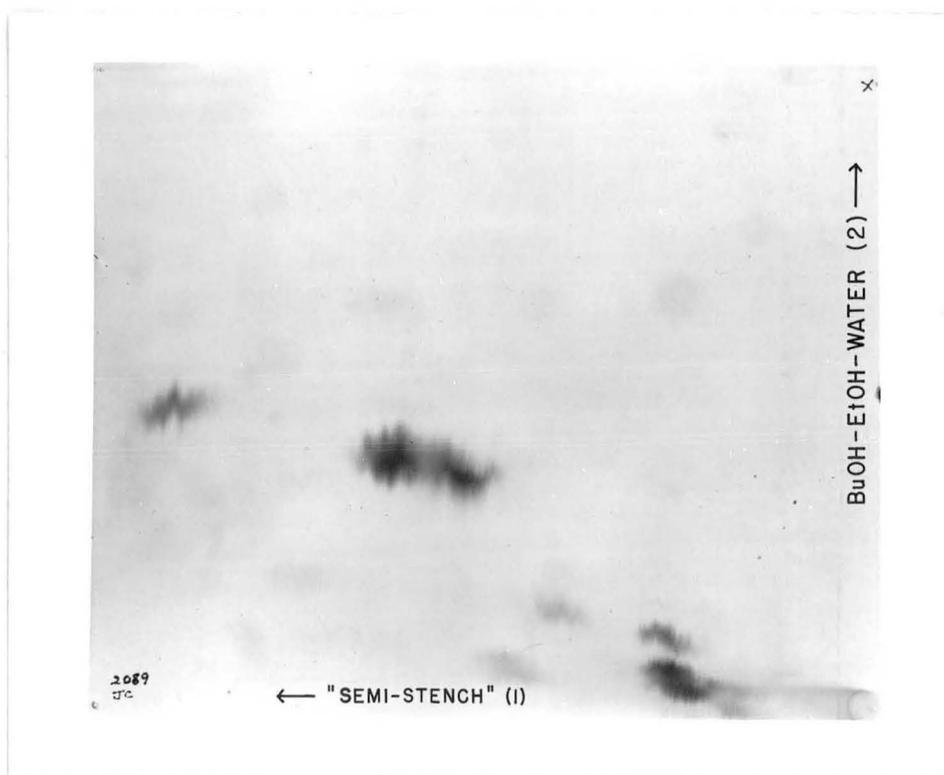


Fig. 2

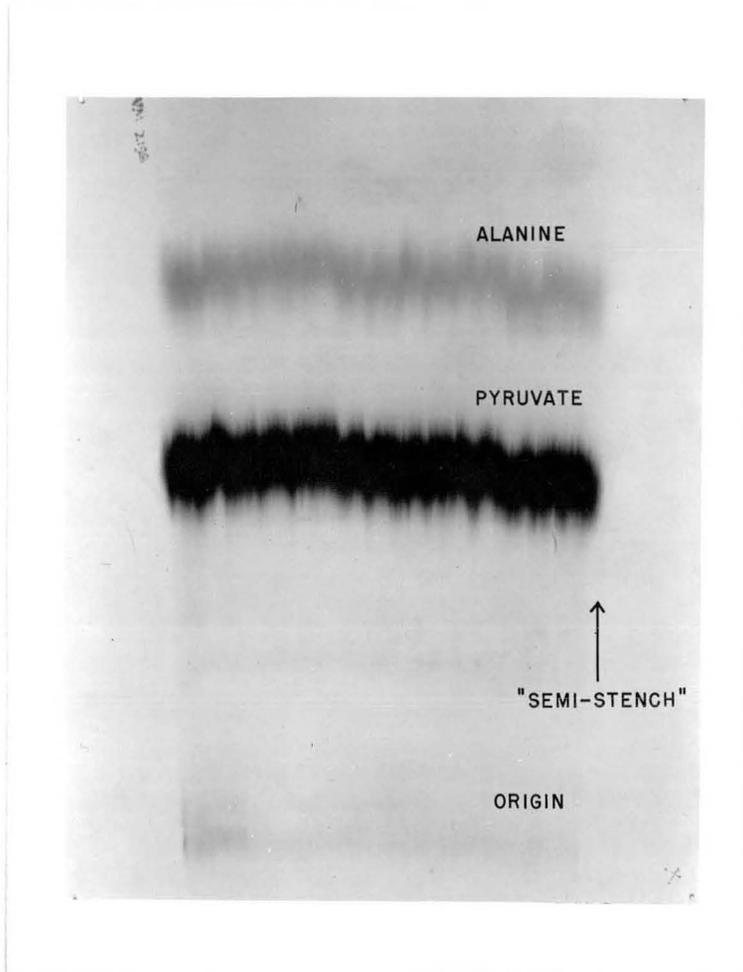


Fig. 3

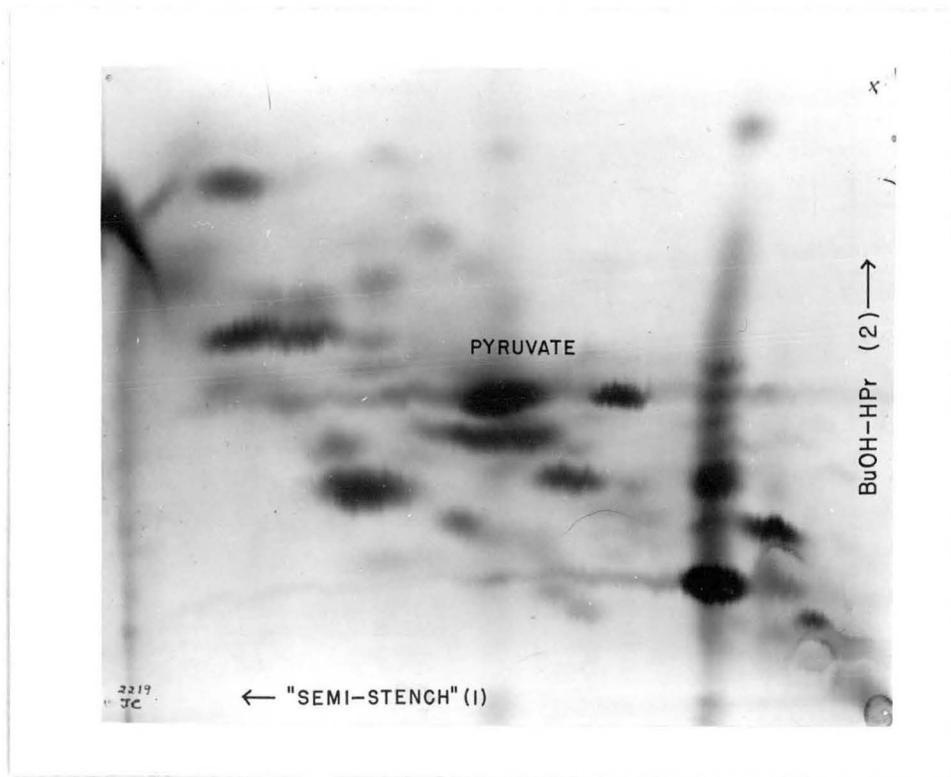


Fig. 4

