the exit pinhole of a G.E. X-ray collimator, and the diffraction pattern is recorded on film in a G.E. flat cassette camera at a specimen-plate distance of 5 cm. Cu radiation (Ni filtered) at 40 kv and 20 Ma is employed. Exposure times are of the order of 4 hrs. Excellent diffraction patterns are obtained in this way from specimens representing not more than 50 μg of material.

Satisfactory, reproducible diffraction patterns have been obtained from a number of amino acids, including the following: *l*-leucine, *dl*-leucine, *dl*-isoleucine, *dl*-norleucine, *dl*-threonine, *l*-threonine, *d*-threonine, glycine, *d*-serine, *dl*-serine, *dl*-allothreonine, and α,γ-diaminobutyric acid dihydrochloride. Unknowns have been successfully identified by comparison with these standard patterns.

This investigation has yielded ample evidence that this method of X-ray analysis can be of tremendous value for the identification of small quantities of crystalline materials separated by chromatography.

References

1. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*, 1944, **38**, 224.
2. POLSON, A., MOSLEY, V. M., and WYCKOFF, R. W. G. *Science*, 1947, **105**, 603.

The Fumarate Content of Certain Tissues of the Rat as Determined by Partition Chromatography

LAWRENCE M. MARSHALL,[1] JAMES M. ORTEN, and ARTHUR H. SMITH

Department of Physiological Chemistry, Wayne University College of Medicine, Detroit

The importance of fumaric acid as a metabolic intermediate in the "citric acid cycle" has become well recognized during the past few years. Progress in this field, however, has been retarded by the lack of a quantitative method for the determination of the small amounts of fumaric acid and certain other related organic acids in the citric acid metabolic cycle. This report presents a new method which has proven satisfactory in our hands for the determination of fumaric acid in the small amounts present in the tissues of the rat and which, in preliminary studies, gives promise of being equally applicable to the simultaneous determination of succinic, malic, citric, and perhaps other organic acids in biological materials.

The present method employs the column partition technique of the English workers (*1, 2*). The method in its present form employs silica gel as an inorganic acid adsorbent which supports mechanically an aqueous phase (0.5 N sulfuric acid) that distributes solutes to a mobile nonaqueous phase (4–10% amyl alcohol in chloroform). The classical distribution law explains the partition that effects the separation of the organic acids in biological materials. The sequence of organic acids liberated by the mobile phase from the vertical silica gel column is directly related to the distribution coefficients of the solutes with reference to the system employed. Citric, malic, lactic, succinic, *fumaric*, β-hydroxybutyric, acetic, and benzoic acids represent a partial list of tested acids in inverse order of their release from the column. Citric acid is, thus, the last of this group to be released. Glutamic, aspartic, and nicotinic acids, like citric, are delivered after fumaric acid. The effluent acids are titrated with 0.004 N sodium hydroxide. The position of fumaric acid in the mobile phase emitted was verified by a supporting method of analysis, devised by us, based on Steenhauer's microqualitative test (*3*) for fumaric acid.

TABLE 1

OBSERVED DISTRIBUTION OF FUMARATE IN POOLED TISSUES OF 12 RATS FASTED FOR 18 HRS

Tissue	No. of determinations	Average fumaric acid measured (μg)	Standard deviation	Average concentration of fumaric acid (mg/100 gm wet tissue)
Brain	2	830	...*	15.0
Kidney	4	404	± 65	9.5
Liver	4	172	± 5.7	7.8
Gastrocnemius muscle	4	127	± 7.8	2.3
Blood	3	16		Less than 0.3

* Range of measured values, 40 μg.

When the fumaric acid emitted from the column is collected in successive small fractions having geometrically increasing volumes (e.g. were the first fraction 1 ml, the next would be 1.13 ml, the next 1.26 ml, etc.), the concentration of fumaric acid plotted against the fraction number (1, 2, 3, 4, etc.) describes a curve which approximates the normal curve of error. When the observed curve is fitted to a theoretical curve for fumaric acid deduced from the binomial law, congruity measures the reliability of the data.

Recovery studies have demonstrated the applicability of these techniques to animal tissues. Data on the fumarate concentrations in several tissues of the fasted adult rat maintained on a stock diet are shown in Table 1. Samples from the pooled tissues of the 12 rats were used.

The foregoing data thus indicate that there is a significant amount of fumaric acid present in the tissues of the rat, particularly in brain, and furnish analytical support for the current concept of the importance of this member of the "citric acid cycle" in metabolic processes. Further studies on other members of the cycle are in progress.

References

1. ISHERWOOD, F. A. *Biochem. J.*, 1946, **40**, 688.
2. MARTIN, A. J. P., and SYNGE, R. L. M. *Biochem. J.*, 1941, **35**, 1358.
3. STEENHAUER, A. J. *Pharm. Weekbl. Ned.*, 1935, **72**, 667.

[1] U. S. Public Health Junior Research Fellow.