A_2. This results in a slight resistance heating of the thermistor. When in position in the ear, the increase in temperature from this source amounts to only about 0.05° C and is constant.

Our use of the instrument has thus far been limited to one individual, and extensive data have not been obtained. The auditory canal temperature was found in this case to be about 0.25° C lower than the sublingual temperature, which it parallels quite closely. The records

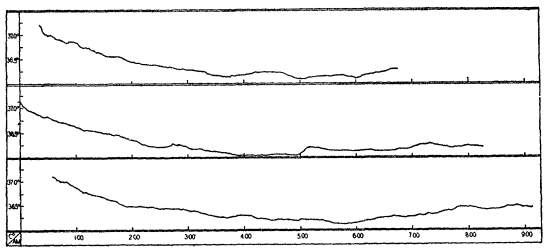

FIG. 3

reproduced in Fig. 3 will serve to illustrate the potentialities of the instrument. Three overnight temperature records are shown. For these measurements it was necessary to place adhesive tape over the ear and ear mold in order to prevent accidental removal during the night. This is unnecessary when the subject is awake. These records were obtained without discomfiture to the subject and without disturbing his sleep.

Use of X-Ray and Electron Diffraction as Methods of Analysis in Biochemical Chromatography

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The resolution and purification of biological materials by partition chromatographic techniques has become well established as a method of biochemical analysis. In particular, the development by Consden, Gordon, and Martin (*1*) of a chromatographic technique which employs strips of wet filter paper as the adsorbent has made possible a very effective method for separation of amino acids. These workers were able to show the presence of 22 amino acids in a single experiment, using a total of only 200–400 μg of sample.

This technique has been employed by several groups in this Laboratory for the separation and identification of amino acids in various biochemical studies. As a result, the problem of independent qualitative identification of the separated constituents of a mixture has become a problem of considerable importance. Various microchemical methods have been used, but the availability of only a few hundred micrograms of purified material makes such analytical techniques difficult.

Physical methods of analysis have also been considered. Among these, electron diffraction appeared to offer many advantages (*2*), the most attractive being its ability to yield diffraction patterns from minute quantities of material. Offsetting this advantage are the well-known difficulties of studying organic materials which are subjected to the bombardment of high-energy electrons. That this difficulty should not be underestimated is perhaps obvious from a casual study of the literature, which reveals a remarkable dearth of publications describing electron diffraction studies of solid organic substances. Nevertheless, it was decided to investigate the technique as a method for qualitative analysis of amino acids separated chromatographically.

A simple method of extraction of small quantities (ca. 200 μg) of an amino acid fraction from the chromatogram was devised. The care customarily exercised in the preparation of specimens for electron diffraction study was observed throughout. The portion of the filter paper containing the desired component of the mixture is cut out and placed in the fold of platinum foil, 1″ square, folded in half. Sufficient distilled water (ca. $\frac{1}{3}$ cc) is dropped on the filter paper to provide a slight excess over the amount absorbed by the paper. After leaching for several minutes, the water solution is dropped on a clean microscope slide or a piece of platinum foil which is immediately placed in a vacuum chamber. Under a moderate vacuum the water freezes rapidly and is removed by sublimation. A residue of minute crystals remains, and this specimen is suitable for study by electron diffraction techniques. This simple method apparently minimizes possible salt formation and contamination of the sample.

Specimens prepared in this way were examined in an electron diffraction camera, using 60-kv electrons and a specimen-plate distance of 65 cm. Considerable difficulty was frequently encountered in obtaining diffraction patterns, despite much effort to standardize very carefully the method of specimen preparation and manipulation. Patterns would appear momentarily and then disappear, a phenomenon which led us to believe that sublimation and/or decomposition of the specimen was occurring. Certain amino acids yielded excellent diffraction patterns with a minimum of effort; others required preparation of many specimens before any pattern could be obtained. The extreme inconsistency of the method was most discouraging, although results, when obtained, were fairly uniform.

While evaluating this situation, the possibility of employing X-ray rather than electron diffraction was discussed. Preliminary experiments were encouraging, and ultimately a very simple technique was developed. The vacuum recrystallized material is scraped from the glass slide and packed in a $\frac{1}{2}$-mm aperture centered in a brass disk 0.4 mm thick. This disk is centered in a recess at

[1] We desire to express our deep appreciation to R. G. Shepherd and P. H. Bell, of the Chemotherapy Division of these Laboratories, for their assistance in providing samples of amino acids for analysis and for advice regarding chemical aspects of the problem.

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.

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

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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.

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