

is the semiquinone radical. The monomethyl-ether of hydroquinone behaves as hydroquinone, which shows that one unsubstituted phenolic hydroxyl group is sufficient for the reaction and corroborates the assumption that the yellow color is not quinone.

There can be no doubt that this colored substance is a free radical existing in a concentration far above the thermodynamically permissible concentration. This experiment shows at least that the free radical of tocopherol is not just a product of paper chemistry but does exist. At ordinary temperatures, it will never accumulate to a directly recognizable extent. On the other hand, its very instability makes it a highly reactive substance.

Although the univalent oxidation may involve a large free energy, the process might be greatly facilitated if tocopherol were a prosthetic group of an enzyme and the process took place intramolecularly within the enzyme-substrate complex. Such an assumption would also fit the fact that the biological activity is restricted to a molecule of specific structure and is not exhibited generally by any mono-substituted hydroquinone.

It is not claimed that the problem as to the mechanism of the action of tocopherol either as a vitamin or as an antioxidant is entirely solved herewith. But the fact that the finding of the semiquinone places tocopherol in the class of the reversible oxidation-reduction systems establishes the analogy to various other vitamins which act as coenzymes or prosthetic groups of enzymes concerned with specific dehydrogenations, namely, riboflavin, nicotinic acid, and thiamine.

References

1. BACHARACH, A. L., and DRUMMOND, J. C. (Eds.). *A symposium on vitamin E*. London: Society of Chemical Industry, 1939.
2. LEWIS, G. N., and LIPKIN, D. *J. Amer. chem. Soc.*, 1942, **64**, 2810.
3. MICHAELIS, L. *Ann. N. Y. Acad. Sci.*, 1940, **40**, 39.
4. MICHAELIS, L. *Amer. Sci.*, 1946, **34**, 573; in *Science in Progress*, 1947.
5. MICHAELIS, L., SCHUBERT, M. P., REBER, R. K., KUCK, J. A., and GRANICK, S. *J. Amer. chem. Soc.*, 1938, **60**, 1678.
6. MORTON, R. A. *The application of absorption spectra to the study of vitamins*, etc. (2nd ed.) London: 1942.

Technique for Making Spreads of Omentum from Small Animals¹

Joy Barnes Cross

Department of Preventive Medicine and Public Health,
Medical Branch, University of Texas, Galveston

Possibly one outcome of the work of Knisely's group (2) on sludged blood will be a re-awakened interest in the potential usefulness of the omentum in studies of the vascular system. Because the omentum is very thin, sectioning is unnecessary for microscopic studies, and so the observer can secure an unmutated and complete survey of the vascular bed over a relatively wide field (1).

Smooth and sizable spreads of this tissue, however, are not easily obtained because as soon as the anchorage

of the omentum to the intestine is destroyed, the mesentery tends to contract and wrinkle badly. The following technique routinely produces smooth spreads of omentum from mice (half grown to adults in size).

The tissue should be taken as soon after death as possible. The use of a duoloupe or dissecting microscope is advantageous. The intestine should be spread out so that a loop bounds a fan-shaped portion of the omentum, avoiding the region of the pancreas if very thin preparations are desired. A slip of cigarette paper (cut crosswise in about thirds) is placed on a gloved fingertip and inserted under the chosen portion of the omentum, retaining the fingertip beneath the omentum until the preparation is placed on a slide. The membrane is spread smoothly against the paper. Unless too much time has elapsed since the death of the animal, the albuminous substances in the tissue will affix it to the paper. The intestinal loop and enclosed "fan" are severed by fine scissors. The cut loop is carefully trimmed away from the enclosed membranous "fan" by cutting through the membrane and the supporting paper. The chosen portion of the paper-supported omentum is left balanced on the fingertip and then gently but firmly pressed with the tissue side down against the slide. Using scissors' tips or needles the spread is smoothed before it is covered with a few drops of fixing solution (we used Schaudinn's). After a delay of a second or so, the spread is blotted dry quickly by pressing with several layers of filter paper.

The next step requires speed, care, and skill. With a fine-pointed forceps for lifting the paper and a teasing needle or scissors' tip to hold the tissue against the slide, the cigarette paper is separated and peeled away. The slide with the affixed tissue should be placed in a jar of fixative immediately. Any of the usual techniques for fixing, dehydrating, and staining paraffin sections may be used for completing the preparation. If, as rarely happens, a spread slips from a slide during these later manipulations, the tissue may be salvaged by handling in the customary manner for "loose" celloidin sections.

References

1. CROSS, J. B., and ANIGSTEIN, L. *Amer. J. trop. Med.*, in press.
2. KNISELY, M. H., BLOCH, E. H., ELIOT, T. S., WARNER, L. *Science*, 1947, **100**, 431-440.

Scanning Instrument for Quantitative One-Dimensional Paper Partition Chromatography

L. S. Fosdick and R. Q. Blackwell
Chemistry Department,
Northwestern University Dental School

The method of Consden, Gordon and Martin (3) for separation and identification of amino acids by paper partition chromatography has found wide application and has been extended to other classes of compounds. Various methods for rendering the method quantitative have been described (4, 5, 6, 7, 8, 9, 10, 11). In this laboratory the method developed by Bull and coworkers (2)

¹ Supported in part by grants from the U. S. Public Health Service and the Office of Naval Research.

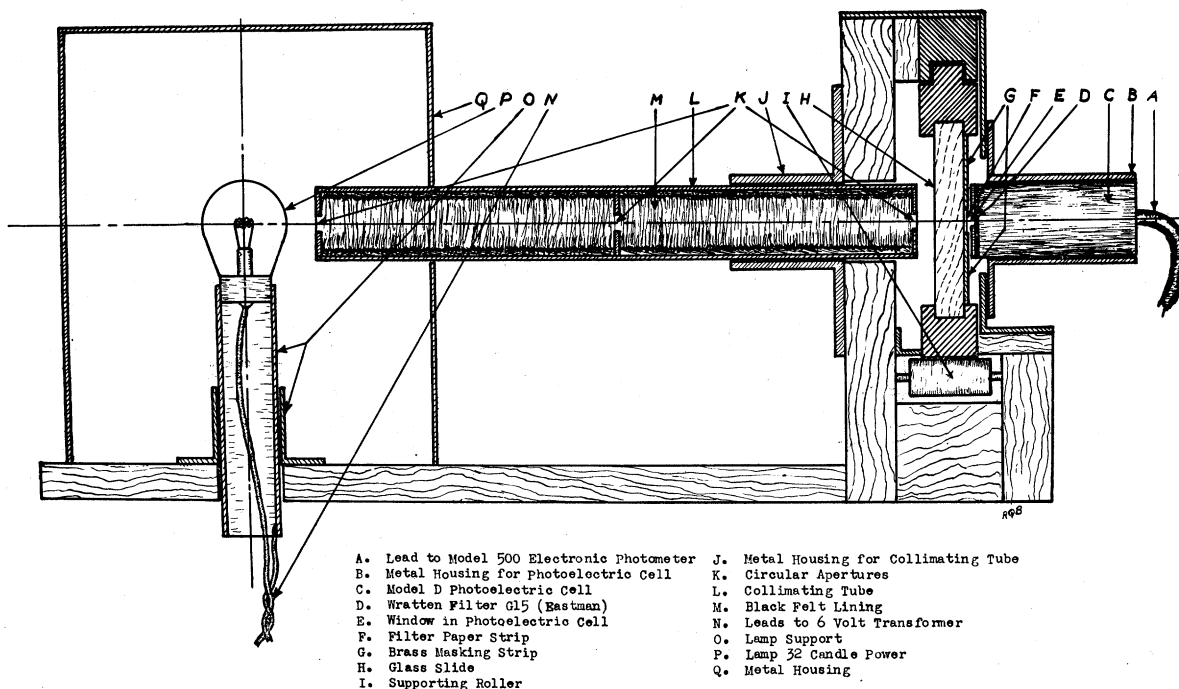


FIG. 1.

on quantitative estimation of amino acids by one-dimensional paper partition chromatography is being used. This method, also developed by Block (1), depends upon the measurement of transmission of light through the filter paper strip containing purple spots developed by the reaction of ninhydrin on the individual amino acids on the strip.

The scanning instrument used in this laboratory for measuring transmission through the paper strips is shown in Fig. 1. The photoelectric cell, C, is connected by lead A to a photometer (Photovolt Corp.). The filter, D, is mounted in E, the cell window. The photoelectric cell fits snugly in the brass housing, B; the optimal distance between the paper strip and the cell can be adjusted by sliding the latter forward or backward in the housing. The housing, B, is held in place by four set screws (not shown) and is adjustable both horizontally and vertically to facilitate positioning the cell window, E, exactly in line with the collimated light beam passing through the paper strip, F. The glass slide, H, mounted in metal strips, moves horizontally on a series of supporting rollers, I. Brass strips, G, mask the entire surface of the glass slide except a narrow portion along the middle of the face of the slide covered by the filter paper strip, F. The light source, P, mounted on the vertically adjustable support, O, is connected by leads, N, to a 110 × 6-v transformer (not shown). The cylindrical housing, Q, encloses the light source. The cylindrical brass collimating tube, L, fits in the brass housing, J, and can be moved forward or backward for optimal adjustment. The three circular apertures, K, in the collimating tube consist of steel washers fitted into the tube. The inside of the collimating tube is lined with nonreflecting black felt, M. A scale, 70 cm in length, graduated from 0 to 100, is in-

scribed on the instrument frame adjacent to the top of the slide, and an indicator line on the slide frame is used to read the scale. In practice all strips are run in the vapor chamber to a total distance of 70 cm (i.e., a distance equal to the length of the scale on the scanning instrument). The color is developed on the strips (2) which are then fastened in the proper position on the glass slide between the brass masking strips. Transmission readings are taken at intervals of $\frac{1}{2}$ scale units (1 scale unit = 0.7 cm) by moving the slide. The transmission readings from the Photovolt meter are plotted directly upon semilog paper as a function of distance in scale units, and the curves treated as described by Bull (2). Using this method the R_f value (3) can be read directly from the plot.

Accuracy of determinations of amino acids using color development with ninhydrin by Bull's method (10) vary for us from 5 to 15% depending upon the amino acid.

References

1. BLOCK, R. J. *Science*, 1948, **108**, 608-9.
2. BULL, H. B., HAHN, J. W., and BAPTIST, V. H. *J. Amer. chem. Soc.*, in press.
3. CONSDEN, R., GORDON, A. H., and MARTIN, A. J. P. *Biochem. J.*, 1944, **38**, 224-32.
4. FISHER, R. B., PARSONS, D. S., and MORRISON, G. A. *Nature, Lond.*, 1948, **161**, 764-5.
5. FLOOD, A. E., HIRST, E. L., and JONES, J. K. N. *Nature, Lond.*, 1947, **160**, 86-7.
6. GOLDBERG, H. J. V., GILDA, J. E., and TISHKOFF, G. H. *J. dent. Res.*, 1948, **27**, 493-496.
7. HAWTHORNE, J. R. *Nature, Lond.*, 1947, **160**, 714-15.
8. KESTON, A. S., UDENFRIEND, S., and LEVY, M. *J. Amer. chem. Soc.*, 1947, **69**, 3151-2.
9. NAFTALIN, L. *Nature, Lond.*, 1948, **161**, 763.
10. TERRILL, P. M. Master's Thesis, Northwestern University, 1948.
11. WOIWOOD, A. J. *Nature, Lond.*, 1948, **161**, 169.