factory agent commercially, it may materially assist in solving one of the important theoretical problems of anesthesia.

References

1. LAWRENCE, J. H., *et al. J. Physiol., London,* **105**, 197 (1946–47).
2. LAZAREV, N. V., LYUBLINA, E. I., and MADASSKAYA, R. Y. *J. Physiol. U.S.S.R.,* **34**, 131 (1948). Abst. from *Leningrad Research Inst. Ind. Hyg. Fiziol. Zhur.*
3. BUTLER, T. C. *J. Pharmacol. Exptl. Therap.,* **98** (Suppl.), 121 (1950).
4. JONES, H. B. In Glasser, O. (Ed.), *Medical Physics,* Vol. II. Chicago: Year Book Pub. (1950).
5. TOBIAS, C. A., *et al. J. Clin. Invest.,* **28**, 1375 (1949).

A Simple Technique for the Identification of Raffinose and Sucrose by Enzymatic Hydrolysis on Paper Chromatograms and the Subsequent Separation of the Hydrolyzed Products by Paper Chromatography

Kenneth T. Williams and Arthur Bevenue

Western Regional Research Laboratory, Albany, California, of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, USDA

During studies involving the identification of sugars by paper chromatography it became necessary to verify the presence of raffinose and sucrose. The small amount of materials available necessitated a minimum amount of handling. The elution of these sugars from the chromatograms, together with their subsequent hydrolysis and concentration before rechromatographing, was undesirable. A new technique was therefore developed.

Because of the relatively rapid rate of hydrolysis of raffinose and sucrose with the enzyme invertase, it was decided to spot the sugar solution on the paper chromatogram and then superimpose the enzyme solution on the sugar spot. It was found that a microliter of solution containing 10–50 μg of raffinose or sucrose could be spotted on the paper, and partial hydrolysis of the sugar could be obtained by superimposing an equal volume of invertase solution on the sugar spot.

By experiment it was found that, if 1 μl of sugar solution was spotted on the paper, 5 μl of invertase solution immediately superimposed on the sugar spot, and the paper allowed to lie for 5 min, the hydrolysis of raffinose and sucrose was complete. The products of hydrolysis could then be partitioned as usual. The chromatographic technique was essentially that used by Partridge (*1*) and McCready *et al.* (*2*). The enzyme preparation was Difco Invertase Solution (for analytical use).[1] The chromatograms were made on Whatman No. 1 filter paper, and an *n*-butanol-ethanol-water (10–1–2) mixture served as the partitioning solvent. When the chromatogram was sprayed with the dinitrosalicylate reagent (0.5% 3,5-dinitrosalicylic acid in 5% NaOH), the melibiose and levulose from the raffinose and the dextrose and levulose from the sucrose were found to be adjacent to known pure sugars used as controls.

When the invertase solution was superimposed on only half of each of the raffinose and sucrose spots, 5 min allowed for hydrolysis, and partitioning carried out in the usual manner, additional information was obtained. When the resorcinol spray reagent (0.1% resorcinol in 0.7 *N* HCl) was used, this chromatogram revealed one half of the original raffinose spot and a part of the original sucrose spot, representing the portions of the original sugars that had not been treated with the invertase solution. In addition, levulose spots also appeared on this chromatogram as a result of the partial enzymatic hydrolysis of the original raffinose and sucrose spots. All three sugars were adjacent to known pure sugars used as controls without the addition of invertase.

This technique is being applied to sugar extracts from plant materials. It should be applicable to systems other than invertase-raffinose-sucrose.

[1] Mention of manufacturers and commercial products does not imply that they are endorsed or recommended by the Department of Agriculture over others not mentioned.

References

1. PARTRIDGE, S. M. *Nature,* **158**, 270 (1946).
2. MCCREADY, R. M., WALTER, E. D., and MACLAY, W. D. *Food Technol.,* **4**, 19 (1950).

Steroid Changes in Incubating Adrenal Homogenates[1]

Carl S. Vestling and Gene F. Lata[2]

Division of Biochemistry, Noyes Laboratory of Chemistry, University of Illinois, Urbana

The presence of a high concentration of cholesterol in the cortex of the adrenal gland suggests an important function. It is known that under conditions of shock or administration of ACTH the adrenal cholesterol falls sharply with a concomitant increase in the release of steroids from this gland (*1*). Evidence has accumulated to indicate that cholesterol is a source compound for cholic acid (*2*), cholestenone (*3*), and progesterone. With this background it became desirable to test further the possible conversion of cholesterol to other steroids under *in vitro* conditions. The work was done throughout with whole homogenates of guinea pig adrenals. Total cholesterol was determined by a micromethod[3] devised during the course of these investigations (accuracy ±6%). Steroids were followed by use of the Zaffaroni procedure (*4*), together with the ultraviolet scanner described by Haines and Drake (*5*).

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[2] From a thesis submitted by Gene F. Lata in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate College of the University of Illinois, 1950. Present address: Department of Biochemistry, State University of Iowa, Iowa City.

[3] To be published.