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

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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

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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.

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Steroid Changes in Incubating Adrenal Homogenates[1]

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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*).

[1] The authors wish to express their appreciation to the Upjohn Company, Kalamazoo, Mich., for research grants in support of this work.

[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.

The tissue preparations were made by rapidly removing the entire adrenals from female guinea pigs and homogenizing them with sufficient chilled water in a Potter-Elvehjem homogenizer to make "15%" preparations. Four-tenths ml of such a homogenate was then incubated in a 10-ml Erlenmeyer flask at 37° C, with continuous shaking for 3 hr with the following additions: 1.6 ml of Krebs-Ringer modified buffer (pH 7.4); 0.2 ml of *ca.* 1×10^{-4} *M* cytochrome *c*;[4] 0.2 ml 0.5% DPN;[4] and 0.6 ml double-distilled water. During incubation a stream of wet oxygen was passed through the chain of flasks. Initial values were obtained by analysis of unincubated flasks, prepared as were the incubated ones, but sampled for analysis

TABLE 1

EFFECT OF INCUBATION ON ADRENAL HOMOGENATES

Description	Cholesterol (γ/flask)	Appearance of "steroid" on strip
Unincubated	1,485	Absent
"	1,640	"
Incubated	948	Present
"	948	"
"	1,070	"

immediately following the additions. In one series a portion of homogenate was heated on a steam bath for 3 min, brought to a boil, cooled, and then used in the incubation flasks as usual. The addition of α-ketoglutarate, acetate, glucose, or enolphosphopyruvate as energy sources did not lead to greater changes in total cholesterol than those reported below.

In Table 1 are shown the results of incubation of unheated homogenates. The paper strip chromatograms, all of which had been developed for 33 hr with benzene: formamide (*4*), showed, for the incubated flasks, quenching of fluorescence at a spot about 2 cm from the origin, as well as at the origin. Strips corresponding to the unincubated flasks showed only the spot at the origin.

Comparison of the chromatograms obtained with those reported by Zaffaroni (*4*) suggested Compounds E and F as two possibilities for the identity of the unknown spot. Samples of these two compounds were obtained from W. J. Haines, of the Upjohn Laboratories, and their chromatographic behavior compared with that of the spot obtained in incubated homogenates. The results of such an experiment are shown in Table 2.

There is, therefore, a 33% fall in cholesterol accompanied by the appearance of another material

[4] Unpublished results of C. S. Vestling and E. D. Nielson indicate a requirement of adrenal homogenates for added cytochrome *c* and DPN in order to get an efficient conversion of fructose diphosphate to pyruvate.

TABLE 2

Description	Cholesterol (γ/flask)	Appearance of steroid on chromatogram (distance from origin)
Unincubated	1,650	Very faint (1.5 cm)
"	1,610	" " (1.5 ")
Incubated	1,060	Clearly present (1.6 cm)
"	1,030	" " (1.8 ")
"	1,200	" " (1.2 ")
Compound E	—	Clearly present (8.5–11.0 cm)
Compound F	—	Clearly present (1.5–4.5 cm)

tentatively identified as a steroid in larger quantities than present in the unincubated adrenal preparations. When the paper strips were treated with ammoniacal silver nitrate (*4*), a faint darkening was obtained at the same positions that were seen to quench fluorescence.

To test the enzymatic nature of these changes, the incubations were repeated, using heated homogenates. The results of such an experiment are shown in Table 3.

TABLE 3

Description	Cholesterol (γ/flask)	Appearance of steroid on chromatogram
Unheated, incubated	637	Present
Unheated, unincubated	800	Absent
Heated, incubated	846	"

The data clearly show a disappearance of cholesterol under the conditions described and are suggestive of the elaboration of a Compound F-like substance accompanying this change. The changes observed are presumably enzymatic and take place under oxidative conditions, comparable to those employed by Sweat, Samuels, and Lumry (*6*) in their work on testosterone conversion. It is emphasized that the steroid elaborated has been characterized so far only by comparison of its chromatographic behavior with known Compounds E and F, upon which basis it resembled the latter. The possibility of an unknown steriod showing chromatographic behavior similar to that of Compound F is, of course, not to be excluded at this time.

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