

163–64°, and was identified as *epi*-smilagenin by a comparison of the mixed melting point with an authentic specimen. Product (A) was similarly identified as smilagenone.

In this connexion it may be mentioned that Marker *et al.*² showed that dehydrotigogenone (diosgenone) when administered to a dog on biscuit diet gave diosgenin, smilagenin and *epi*-smilagenin.

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Thiobarbituric Acid Spray Reagent for Deoxy Sugars and Sialic Acids

RECENTLY, new sensitive assays have been reported for deoxy sugars¹, 2-keto,3-deoxy sugar acids²⁻⁴, and sialic acids^{5,6}. In these assays, the products of periodate oxidation, malonaldehyde from deoxy sugars and β -formylpyruvic acid from the latter two groups of compounds, are coupled with 2-thiobarbituric acid to produce a bright red chromophore. I wish to report an adaptation of these methods for spraying paper chromatograms.

The procedure for the detection of deoxy sugars and 2-keto,3-deoxygluconic acid is as follows. After removal of solvent the paper is sprayed with a 0.02 M aqueous solution of sodium periodate. After 15 min. a solution consisting of ethylene glycol, acetone and concentrated sulphuric acid (50 : 50 : 0.3) is sprayed on to the paper. After 10 min. the paper is sprayed with an aqueous solution of 6 per cent sodium 2-thiobarbiturate. (Although several chemical supply companies sell '2-thiobarbituric acid', we have found that the product is frequently mislabelled and that the product is a salt of 2-thiobarbituric acid. Only 0.9 gm. of the acid is soluble in 100 ml. water, whereas 6.5 gm. of the sodium salt is soluble in the same amount of water. The free acid may be rendered soluble by the addition of an equimolar amount of sodium hydroxide.) Red spots appear after heating at 100° for 5 min. Under an ultra-violet light ('Mineralight') the spots give a red fluorescence. As little as 0.5 μ gm. of 2-deoxyribose can be seen. We have used the spray to detect 2-deoxyglucose, 2-deoxyxylose, and 2-deoxygalactose. Although 3-deoxyglucose can also be detected on paper, the colour intensity of the spot is considerably less than that of an equimolar amount of 2-deoxyglucose.

For the detection of sialic acids a solution of 0.05 M sodium periodate in 0.05 N sulphuric acid is used. The glycol and thiobarbituric acid solutions are the same as above. A longer heating time of about 10 min. is required. Approximately 3 μ gm. of sialic acids can be detected.

Using this procedure, deoxy sugars and sialic acids have been successfully located on Whatman No. 1, 3 and 3 MM paper, using a variety of acidic, basic and neutral solvent systems.

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Enzymatic Deamination of 5-Deoxycytidylic Acid and of 5-Methyl-5'-deoxycytidylic Acid in Growing and in Non-growing Tissues

THE deamination by enzymatic hydrolysis of 5'-deoxycytidylic acid to 5'-deoxyuridylic acid and of CH₃-5'-deoxycytidylic acid to 5'-deoxythymidylic acid has been previously reported in sea urchin egg homogenates^{1,2}, and in adult rabbit liver³.

The deamination of 5'-deoxycytidylic acid and of CH₃-5'-deoxycytidylic acid has been investigated in the developing sea urchin embryo⁴, in normal and regenerating rabbit liver, in adult rats and in embryonic rat tissues, in adult chick and in chick embryos in order to correlate these enzyme activities with biosynthesis of deoxyribonucleic acid and hence with the metabolic activities of tissues that grow by increase in the number of the component cells.

In this preliminary communication some of our results for tissues of warm-blooded animals are reported. These clearly show that the 5'-deoxycytidylic acid and the CH₃-5'-deoxycytidylic acid deaminations occur to a greater extent in growing tissues than in normal non-growing tissues. In adult tissues of rat and of chick it is impossible to detect with our assay the activities in the unfractionated homogenate.

The tissues, as soon as they were removed from the animal, were homogenized in a Potter-Elvehjem glass homogenizer with phosphate buffer. The homogenate was centrifuged for 15 min. at 4° C. and at 18,000g. The supernatant was used for the enzymatic assay.

The enzymatic assays are based on the ultra-violet spectral variations caused by the deamination. Tables 1 and 2 show the assays. All the enzymatic determinations have been made in the range in which

Table 1. ASSAY OF 5'-DEOXYCYTIDYLIC ACID DEAMINASE

Time (min.)	Absorbance at :			
	232 m μ	250 m μ	267 m μ	280 m μ
0.5	0.120	0.175	0.440	0.530
6	0.120	0.195	0.445	0.495
11	0.115	0.215	0.440	0.450
ΔA	-0.005	+0.040	0	-0.080

Centrifuged supernatant of a homogenate from chick embryo's liver: protein, 2 mgm.; 5'-deoxycytidylic acid, 1 μ mole; *tris*, 30 μ moles. Final volume, 0.5 ml.; pH 7.2. Incubation at 38° C. At the indicated times 0.1 ml. was removed and 0.1 ml. 10 per cent perchloric acid was added. After high-speed centrifugation 0.1 ml. of the clear colourless supernatant was diluted with 2.4 ml. of 0.01 N hydrochloric acid, and the absorbance of this solution was determined with a Beckman DU spectrophotometer.