for detecting microgram quantities of acid on the spot plate.

- One hundred milligrams of Dee-O was dissolved in 10 ml of acetate buffer of pH 5.3 to 5.6, and about 1 g of scraped raw potato pulp was added. After it had stood 15 to 20 minutes, the reagent was filtered through glass wool. The raw potato is a convenient, essentially glucose-free source of peroxidase.
   When o-tolidine dihydrochloride was used, 1 g
- 7. When o-tolidine dihydrochloride was used, 1 g was stirred with 0.45 g of KOH (85 percent pure) in 100 ml of 95-percent ethanol until it was dissolved, and the precipitated KCl was removed by filtration.

12 December 1956

## Infrared Spectra of Mixtures of a- and $\beta$ -D-Glucose Pentaacetate

Infrared spectra have been extensively used to identify sugars and their derivatives (1). The  $\alpha$ - and  $\beta$ -anomers can also be differentiated through the presence or absence of certain characteristic peaks in their infrared spectra (2). In connection with some studies undertaken in this laboratory, it was proposed to use the intensity of an absorption peak characteristic of a particular anomer to determine the concentration of that anomer in solution.

In order to see whether or not this was feasible, we first investigated the infrared spectra of mixtures of the two anomers,  $\alpha$ -D-glucose pentaacetate—mp. 112°C;  $[\alpha]^{21}$ D, + 101° (EtOH, c., 0.5) (3)—and β-D-glucose pentaacetate-mp, 133 - $134^{\circ}C; [\alpha]^{21}D, +2^{\circ}$  (EtOH, c., 0.5) (3, 4)-in chloroform and acetone solutions. The  $\alpha$ - and  $\beta$ -D-glucose pentaacetates were mixed in the proportions  $1 \alpha/4 \beta$ ,  $1 \alpha/1 \beta$ , and  $4 \alpha/1 \beta$  at a total concentration of 1.00 percent (wt./vol.), and the infrared spectra were recorded with a Beckman IR3 recording spectrophotometer. This instrument permits one to obtain infrared spectra of compounds in solution without the spectrum including any contributions from the solvent.

Table 1 shows a series of absorption peaks found in the 8- to 15-µ region. The majority of those shown are apparently diagnostic of either the  $\alpha$ - or  $\beta$ -anomer. The intensity of absorption is indicated by the figures in parentheses, which represent the percentage absorption, taking the absorption at 5.68  $\mu$  as 100 percent. The absorption at 5.68  $\mu$ was the same for both the anomers and their mixtures and was one of the most intense bands. There are also shown, for comparison, two peaks that are found in both  $\alpha$ - and  $\beta$ -D-glucose pentaacetate. The α-anomer shows a slightly more intense absorption at 8.7 µ than the  $\beta$ -anomer. Both the anomers show essentially the same absorption at 11.15  $\mu$ .

Certain absorption peaks, such as that at 8.95 µ which is apparently characteristic of the  $\beta$ -anomer or that at 9.86  $\mu$ which is apparently characteristic of the  $\alpha$ -anomer, decrease in intensity as the concentration of the anomer is decreased. On the other hand, the absorption peak at 9.65 µ which is characteristic of the  $\beta$ -anomer or the peak at 11.00  $\mu$  which is characteristic of the *a*-anomer rapidly disappears in the presence of the opposite anomer. It should be noted that the intensity of absorption is apparently not a factor. The absorption at 9.65  $\mu$  is very intense, while that at  $11.00 \ \mu$  is rather weak. Other examples will be apparent from Table 1. Essentially the same spectra were obtained when the solvent was allowed to evaporate and the solids run as films.

Since certain of the absorption peaks characteristic of an anomer behave as might be expected, and since the optical rotation of the mixtures was as calculated, we do not believe that there has been any interconversion between the  $\alpha$ and  $\beta$ -anomers. A possible explanation is that an association takes place in solution

Table 1. Infrared absorption at several wavelengths of chloroform or acetone solutions of  $\alpha$ - and  $\beta$ - and mixtures of  $\alpha$ - and  $\beta$ -D-glucose pentaacetate. Total concentration, 1.00 percent (wt./vol.); wavelength in microns. The figures in parentheses represent the amount of absorption as compared with the absorption at 5.68  $\mu$  (percentage). The adsorption at 5.68  $\mu$  was the same for each anomer and for the mixtures.

β	$4\beta/1\alpha$	$1\beta/1\alpha$	$1\beta/4\alpha$	α
8.70 (43)	8.65 (43)	8.65 (60)	8.65 (60)	8.65 (65)
8.95 (55)	8.95 (53)	8.95 (46)	8.90 (42)	
<b>9.</b> 65 (92)	9.62 (94)	<b>x</b> <i>y</i>	• •	
	9.85 (50)	9.87 (73)	9.87 (73)	9.86 (78)
10.15 (26)	10.15 (26)	10.18 (25)	10.15 (25)	10.15 (24)
			10.35 (16)	10.35 (18)
10.43 (21)	10.45 (18)	10.45 (19)		• •
10.65 (62)	10.65 (49)	10.65 (53)	10.65 (23)	
10.75 (52)	10.75 (41)	10.75 (44)	10.80 (24)	
			11.00 (35)	11.00 (37)
13.45 (9)	13.45 (7)	13.45 (8)		· · /
	14.05 (6)	14.05 (5)	14.15 (10)	14.17 (13)
14.45 (11)	14.45 (10)	14.42 (9)		· · ·

between the  $\alpha \text{-}$  and  $\beta \text{-}anomers$  that suppresses the vibrations responsible for the infrared absorption characteristic of the anomer.

It would seem that considerable care should be taken when infrared analysis is used either for the identification or quantitative determination of sugars, particularly if there is a possibility that anomers are present (5).

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- 5. This report is published with the permission of the Bureau of Ordnance, Navy Department. The opinions and conclusions are those of the authors.

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## Glycolysis by Tumor Mitochondria and the Action of Insulin

The cardinal role of glycolysis (formation of lactic acid from glucose) in the metabolism of living cancer cells has been recognized for well over 30 years (1). A high rate of glycolysis may also be readily demonstrated in cell-free homogenates prepared from tumors. However, the view is still widely held, despite evidence to the contrary (2), that the glycolytic enzymes of tumor cells (and of normal cells) are localized primarily in the nonparticulate fluid fraction of the cell cytoplasm. Mitochondria are regarded, at best, as potential stimulators of the glycolysis of the fluid fraction or else as playing some obscure part in the integrated functioning of the combined cell fractions. Mitochondria are generally considered not to possess the full complement of enzymes required to convert glucose into lactic acid.

During the course of investigations on hormonal regulation of the subcellular glycolysis of tumors, we have obtained new evidence of high intrinsic rates of anaerobic glycolysis by tumor mitochondria, provided that the mitochondria have been supplemented with supernatant fraction that has been enzymatically inactivated. Thus, when the supernatant fraction of a tumor homogenate