any signs of illness. This synergism between bacteria and metabolic inhibitor has been confirmed in several duplicate experiments not only with the same pathogen and inhibitor but with other pathogens and poisons as well. These results have broad implications for the phenomenon of parasitism and should be investigated from a variety of approaches. More detailed reports are in press.

References

1. BERRY, L. J., and MITCHELL, R. B. USAF School of Aviation Medicine Project No. 21-35-005, Repts. No. 1, 2, 5, 7, 8. (1951 and 1952.)
2. KALTER, S. S., and TEPPERMAN, J. *Science,* **115**, 621 (1952).
3. ACKERMANN, W. W. *J. Exptl. Med.,* **93**, 635 (1951).
4. AINSLEE, J. D. *Ibid.,* **95**, 9 (1952).
5. PETERS, R. A., and WILSON, T. H. *Biochem. et Biophys. Acta,* **9**, 310 (1952).
6. BUFFA, P., and PETERS, R. A. *J. Physiol.,* **110**, 488 (1950).
7. QUASTEL, J. H., and WHETHAM, M. D. *Biochem. J.,* **19**, 520 (1925).
8. PARDEE, A. B., and POTTER, V. R. *J. Biol. Chem.,* **178**, 241 (1949).
9. BUSCH, H., and POTTER, V. R. *J. Biol. Chem.,* **198**, 71 (1952).
10. LIFSON, N., and STOLEN, J. A. *Proc. Soc. Exptl. Biol. Med.,* **74**, 451 (1950).

Manuscript received December 17, 1952.

Identification of Glycogen in Whole Bacterial Cells by Infrared Spectrophotometry

Seymour Levine,[1] Heber J. R. Stevenson, and Robert H. Bordner

Environmental Health Center, Public Health Service, Cincinnati, Ohio

A recent report of infrared spectra of intact bacterial cells indicated that some of the bands could be interpreted as protein, nucleic acid, and polysaccharide absorptions (*1*). Subsequent work has shown, in addition, that a glucan of the glycogen-starch type can be identified in spectra of whole cells. This glucan was isolated and proven to be glycogen.

Absorption bands at 8.7, 9.25, and 9.75 μ were observed in the spectra of dry films of enteric bacteria grown on nutrient agar with added carbohydrate. When the growth medium lacked the carbohydrate, only the 9.25-μ band was observed in this spectral region (Fig. 1). The effect of the carbohydrate on the bacterial spectrum was more pronounced when cultures were incubated at 15–20° C rather than at 37° C. The additional absorption bands at 8.7 and 9.75 μ, when weak, appeared only as inflections on the sides of the 9.25-μ band, whereas, when strong, they were accompanied by a deepening of the 9.25-μ band and the appearance of new absorptions at 10.75, 11.8, 13.2, and 14.2 μ.

In an attempt to find the origin of this group of absorption bands, cells of *Aerobacter aerogenes* grown at 15–20° C for 2 days on nutrient agar (Difco) with 1% glucose were subjected to chemical fractionation; infrared spectrophotometry was used to follow the course of the procedure (Fig. 1). Since aqueous extraction failed to remove the characteristic bands from the bacterial spectrum, the cells were disintegrated by sonic vibrations (10 kc). After the debris was removed by centrifugation, infrared spectra showed the desired material in the opalescent supernatant. It was found to be precipitable by 1 volume of 95% ethanol, and the spectra revealed that successive reprecipitations caused progressive attenuation of the 6.05- and 6.45-μ bands which can be attributed primarily to protein. The last traces of protein were removed by shaking with chloroform and isoamyl alcohol. After this treatment the spectrum had no band at 6.45 μ and only a weak band at 6.05 μ, the latter probably due to residual water. The spectrum of the final product showed the characteristic bands at 8.7, 9.25, 9.75, 10.75, 11.8, 13.2, and 14.2 μ, and it was identical with the spectrum of a commercial glycogen preparation. The

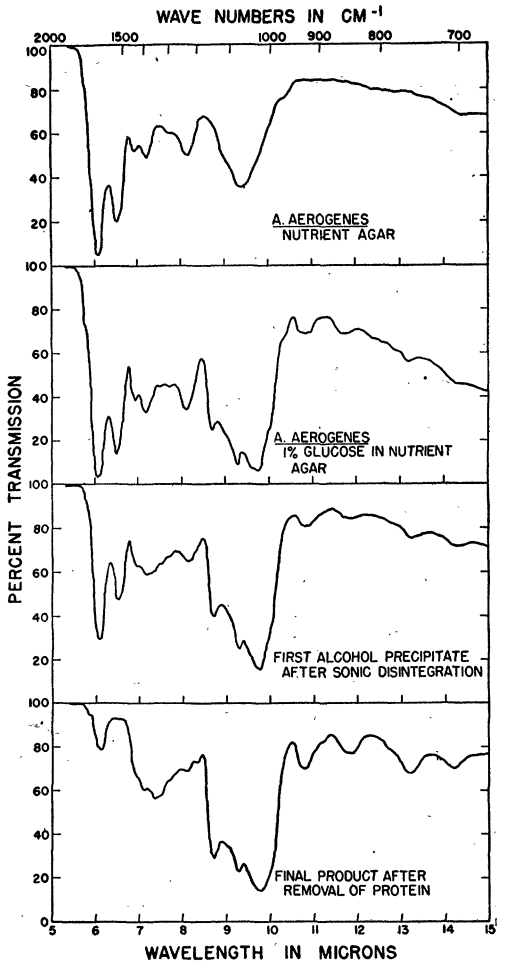


FIG. 1. Infrared spectra showing presence of glycogen in glucose-grown cells.

[1] Present address: Department of Pathology, University Hospital, New York University-Bellevue Medical Center, New York.

product gave a reddish-brown coloration with iodine which is typical of glycogen.

The spectra of whole cells can now be used to follow the course of glycogen production and destruction under various conditions of growth. The amount of this polysaccharide in the bacterial cell can be estimated from the intensity of the 9.75-μ glycogen band. This is compared to the 6.45-μ protein absorption band which acts as an internal standard. The method has the following limitations: the absorption of small amounts of glycogen may be masked by bands due to other materials, particularly slime-layer or capsular polysaccharides; and the spectrum of glycogen resembles closely the spectrum of starch or dextrin. The advantages of the method are that it is rapid and simple, it involves no chemical manipulations, and it requires very small samples (as little as 0.4 mg of dried cells) (*2*). Later observations on the occurrence of glycogen in bacteria, as well as chemical and ultracentrifugal studies of this polysaccharide, will be reported.

References

1. LEVINE, S., STEVENSON, H. J. R., CHAMBERS, L. A., and KENNER, B. A. *J. Bacteriol.*, **65**, 10 (1953).
2. STEVENSON, H. J. R., and LEVINE, S. *Rev. Sci. Instr.*, **24**, 229 (1953).

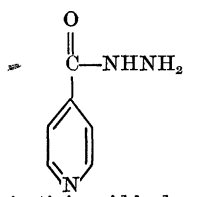
Manuscript received February 17, 1953.

Micro Determination of Isoniazids in Blood

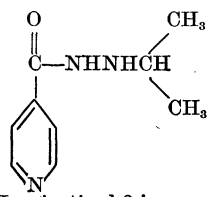
Morris B. Jacobs

Bureau of Laboratories,
Department of Health, New York City

Compounds like isonicotinic acid hydrazide and the isopropyl derivative of isonicotinic acid hydrazide (1-isonicotinyl-2-isopropyl hydrazine) have been approved for use in the treatment of tuberculosis under medical supervision by the U.S. Food and Drug Administration. Accurate and sensitive methods for the determination of these compounds in blood would be valuable in following the course of treatment. Such methods have been proposed by Rubin *et al.* (*1*) and by Kelly and Poet (*2*). These methods are, however, cumbersome and do not lend themselves readily to the ordinary techniques of a clinical laboratory. The monaqueous titration method of Alicino (*3*) and the iodometric methods of Canbäck (*4*) and of Alicino are not applicable.



Isonicotinic acid hydrazide



1-Isonicotinyl-2-isopropyl hydrazine

The following method is simple and more rapid than the methods mentioned. It depends upon the reduction of potassium ferricyanide in acid solution by the isoniazid with the subsequent formation of a Prussian or Turnbull's blue and the colorimetric estimation of the greenish color formed from the yellowish of the ferricyanide and the blue of the iron complex compound.

Reagents. Sodium tungstate solution, 10%: Dissolve 10 g of reagent grade, carbonate-free sodium tungstate, $Na_2WO_4 \cdot 2H_2O$ in water and dilute to 100 ml.

Sulfuric acid, 2/3*N*: Tare a 50-ml beaker. Weigh into this beaker 35 g of concentrated sulfuric acid, specific gravity 1.84. Place approximately 500 ml of distilled water into a liter volumetric flask. Pour the sulfuric acid carefully into the liter flask containing the water, while swirling the flask. Cool, complete to volume, and check the normality against standard sodium hydroxide solution. Adjust the concentration, if necessary.

Acetic acid 3*N*: Prepare in the customary manner.

Potassium ferricyanide solution: Dissolve 2 g of analytical, reagent grade potassium ferricyanide, $K_3Fe(CN)_6$, in distilled water and dilute to 500 ml. Store in a brown bottle protected from light and preferably under refrigeration. Transfer small volumes to another brown bottle for daily use or prepare a fresh solution, as desired.

Standards may be conveniently prepared using recrystallized 1-isonicotinyl-2-isopropyl hydrazine phosphate or isonicotinic acid hydrazide and normal horse serum or pooled normal human blood plasma.

Standard isoniazid solutions: Weigh carefully 0.200 g of isoniazid and transfer to a 200-ml volumetric flask. Add distilled water, shake until dissolved, and dilute to volume. This is the stock standard solution and contains 1 mg/ml.

Transfer with the aid of a volumetric pipet 1 ml of the stock standard solution to a 50-ml volumetric flask and dilute to volume with distilled water. This solution contains 20 μg/ml.

Standard curves: Transfer with the aid of pipets 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, and 4.0 ml of the diluted standard isoniazid solution containing respectively, 10, 20, 30, 40, 50, 60, and 80 μg of isoniazid to 1 ml of normal horse serum or pooled normal human blood plasma. Dilute to 6 ml with distilled water and proceed with the method as detailed below. Since only 4 ml of the tungstate filtrate is used, the standard curve represents half the concentrations listed above, namely, 5, 10, 15, 20, 25, 30, 35, and 40 μg, respectively.

Transfer 1 ml of blood plasma to a 16 × 150-mm test tube. Add 5 ml of water, mix; add 1.0 ml of 10% sodium tungstate solution and mix with an individual glass rod; add 1.0 ml of 2/3*N* sulfuric acid, mix with an individual glass rod, and place the tubes in a water bath at 80–85° C for 1 min to coagulate the precipitate. Remove the tubes from the hot bath, chill in a cold water bath, dry the outside of the tubes, and filter