classified as 2A, and one classified as 2B showed increases in the carbon dioxide combining power after exercise. (Table II.) We shall not attempt to explain these increases in the present report.

The results indicate that children whose tolerance for work is greatly diminished show greater changes in the oxygen unsaturation, lactic acid, and carbon dioxide combining power after performing measured amounts of work than children whose exercise tolerance is slightly diminished.

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Chemistry of the Lipoids of Tubercle Bacilli: XV. Water-soluble Sugars Obtained on Hydrolyzing Phosphatides from Human and Avian Tubercle Bacilli.*

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The phosphatide A-3 obtained from the human type of tubercle bacilli, strain H-37,[1] yielded on hydrolysis about 33% of water-soluble material, but when the preliminary analysis was published only 2 of the water-soluble constituents had been identified, *viz.*, glycerophosphoric acid and glucose. Mention was made in the former publication[1] that a slightly soluble phenylhydrazine derivative was obtained from the aqueous solution and this compound was regarded as a phenylhydrazine salt of a sugar acid. In addition, we also obtained a small amount of a colorless crystalline compound from the concentrated syrup. This unidentified substance was more specifically referred to in the paper dealing with the analysis of the phosphatide isolated from the avian tubercle bacilli.[2] We have recently examined more thoroughly the sugar fractions obtained from the phosphatide A-3 as well as those obtained from the phosphatide from the avian bacillus and we have been able to identify 2 other substances which are present in the hydrolysis mixture, *viz.*, mannose and inosite.

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[1] Anderson, R. J., *J. Biol. Chem.*, 1927, lxxiv, 537.

[2] Anderson, R. J., and Roberts, E. G., *J. Biol. Chem.*, 1930, lxxxv, 519.

The separation of the water-soluble constituents was accomplished as follows: After hydrolysis with boiling dilute sulfuric acid the fatty acids were extracted with ether and the aqueous solution was freed of sulfuric acid quantitatively with barium hydroxide. After the solution had been filtered it was concentrated under reduced pressure, neutralized with barium hydroxide, and the barium phosphate and barium glycerophosphate were precipitated by adding alcohol and removed by filtration. A slight amount of barium in the filtrate was removed quantitatively with sulfuric acid and the solution was concentrated to a thin syrup. An excess of phenylhydrazine dissolved in a little alcohol was added when a crystalline derivative separated almost immediately. After the crystals had been filtered off the excess of phenylhydrazine was removed from the filtrate by treatment with benzaldehyde. The resulting precipitate was filtered off and the filtrate was extracted with ether. The solution was then concentrated to a syrup in a vacuum desiccator, when some colorless crystals separated. The crystals were freed from adhering syrup by washing with cold dilute alcohol and with 95% alcohol. The filtrate on concentration yielded a syrup which on treatment with phenylhydrazine hydrochloride and sodium acetate gave a good crop of glucosazone crystals.

The slightly soluble phenylhydrazine derivative, mentioned above, was recrystallized from hot 60% alcohol and it was obtained in the form of large dense colorless rhombic plates which were identical with crystals of mannose phenylhydrazone. The substance melted at 193-194° and there was no depression of the melting point when mixed with pure mannose phenylhydrazone. On analysis we found 10.45% of nitrogen, which is in close agreement with the calculated value, namely, 10.37%. The optical properties of the crystals have been compared with those of pure mannose phenylhydrazone by Dr. E. J. Roberts of this laboratory and the properties were found to be identical.

It appears to be well established, therefore, that one of the reducing sugars formed on hydrolyzing the phosphatides from the human and the avian tubercle bacilli is mannose.

The colorless crystalline compound which was isolated from the concentrated syrup has been found to be identical with the ordinary inactive inosite. The crude crystals, which contained a small amount of ash, were purified by several crystallizations from dilute acetic acid by adding alcohol and were obtained in the form of colorless prisms or needles characteristic of anhydrous inactive inosite. The substance gave the Scherer reaction and melted at

224-225° and there was no depression when mixed with some inactive inosite prepared from phytin. The values found on combustion agreed closely with the formula $C_6H_{12}O_6$. The optical properties of the crystals were examined by Dr. E. J. Roberts and were found to be identical with those of the ordinary inactive inosite. The identification of inosite may, therefore, be regarded as fully established.

Both inosite and mannose have been found widely distributed in plant and animal cells but this is the first time so far as we are aware that either substance has been observed as occurring in tubercle bacilli.

In addition to inosite and mannose the water-soluble syrup also contained a considerable amount of another reducing sugar which was believed at first to be ordinary glucose because it gave a good yield of typical glucosazone. The glucosazone, after it had been recrystallized from dilute alcohol, melted with decomposition at 205-206° and there was no depression of the melting point when the substance was mixed with pure glucosazone.

The syrup did not consist of pure glucose, however, because it was levorotatory and when heated with hydrochloric acid and resorcinol, a bright red coloration characteristic of ketoses was obtained. The rotation was determined on a solution containing 0.4603 gm. of the dried syrup in 25 cc. of water. In a 2 dm. tube the observed rotation was —0.38°; hence $[\alpha]^{22}_D = 10.3°$. It is probable, therefore that the residual syrup contained a mixture of glucose and fructose similar to invert sugar.

So far as we can judge by the results obtained, the 3 substances, mannose, inosite and invert sugar are present in the syrup from both phosphatide preparations in about equal amounts. We have no data to indicate in what manner these sugars are combined in the molecules of the phosphatides. It should be mentioned, however, that the phosphatides contain no free reducing sugar and it appears probable therefore that the simple carbohydrates which are obtained after complete hydrolyses are combined in the original molecules in the form of a polysaccharide which in turn must be combined either with fatty acids or with glycerophosphoric acid.