A method for the determination of free myo-inositol in biological fluids and tissues

Microbiological methods are generally used for the measurement of the myo-inositol content in biological material. More recently, methods have been proposed involving the use of an enzyme prepared from animal tissues[1] or bacteria[2].

During the course of an investigation of the myo-inositol content of adult and fetal fluids and tissues, we have used the chemical method presented in this paper.

It is essentially an adaptation of the general method for sugars described by HIRST AND JONES[3]: myo-inositol is separated from other carbohydrates by paper chromatography, eluted from the chromatogram and oxidized by sodium meta-periodate to yield formic acid, which is then determined titrimetrically.

For routine determinations, the biological material to be analyzed by this method should contain a minimum of 0.3 mg inositiol. The tissues to be analyzed are washed with 0.9 % NaCl at 0° for a few seconds, blotted, placed on solid CO_2 and then weighed while frozen.

Free myo-inositol is extracted from the tissues by grinding in water at room temperature. Proteins are precipitated with $Ba(OH)_2$–$ZnSO_4$ by the method of SOMOGYI[4]. The extract is centrifuged and the supernatant containing free myo-inositol is deionized by passing it through an 0.5 × 6.0 cm mixed-bed ion-exchange resin column (Dowex 50 – X12, H^+; and Dowex 1 – X8, formate$^-$). The eluent is collected in an evaporating dish and dried at 60°. The dry residue is dissolved in water to make up a solution in which inositol has the approximate concentration of 1 μg/μl.

Known amounts of the solution, estimated to contain about 50–400 μg inositol are then chromatographed on 3-cm wide strips of Whatman No. 1 paper by descending chromatography, an extra strip being run for each sample as a marker. The chromatograms are developed with ethyl acetate–pyridine–water (2:1:2). After a 16-h run at room temperature, the strips are dried, the marker strip stained by dipping, with a satd. solution of $AgNO_3$ in acetone and 5 % NaOH in alcohol according to SMITH[5]. By reference to the chromatogram of a standard solution, myo-inositol is easily identified as a slowly developing, dark band close to the origin. Using the stained marker strip as a guide, the area of paper that contains myo-inositol is cut and eluted with 2 ml water. To 1 ml of the eluent, in 10 × 160 mm tubes with screw caps, 0.4 ml sodium meta-periodate (0.25 *M*) are added. These tubes are placed in a water bath at 100° for 20 min. The tubes are then cooled, 0.2 ml of ethylene glycol are added and the formic acid is titrated.

Rather than titrating the formic acid directly, we found it more convenient to neutralize it with NaOH and titrate the excess NaOH with 0.001 *N* H_2SO_4 in the presence of phenol red as the indicator. The procedure adopted is the following. In a 50-ml syringe are placed 50 ml aq. 25 % NaCl and to it are added with a micro-pipette about 17 μl satd. NaOH. In this way a NaOH solution, approx. 0.005 *N*, relatively free of carbonate even after a brief exposure to air, has been prepared. By way of a stainless-steel three-way stopcock, the 50-ml syringe is attached to a 1-ml Krough-Keyes pipette set to deliver approx. 0.5 ml of solution. With this arrangement it is possible to deliver constant amounts of NaOH, rapidly and precisely, from the 50-ml syringe into the test tubes that contain the formic acid. One or more

aliquots of NaOH are delivered until the indicator turns red. The excess NaOH is titrated with the 0.001 N H_2SO_4 from a microburette calibrated to 0.01 ml.

When oxidized by sodium meta-periodate under the conditions described in this method, one mole of myo-inositol yields 4.5 moles of formic acid[3]. As shown in Fig. 1, myo-inositol is fully recovered from the paper after chromatography. The blank due to the paper is on the average 0.2 μequiv. of acid. In a series of 115 duplicate determinations, in which two strips were plated with the same solution and analyzed separately, the average difference in a pair of samples was 0.063 μequiv. of acid, which corresponds to 2.5 μg myo-inositol.

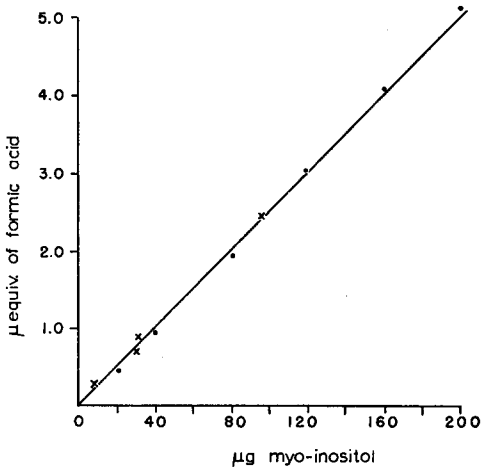

Fig. 1. The diagram represents the relationship between myo-inositol and the formic acid produced by its oxidation. The crosses indicate the results of determinations on known amounts of myo-inositol that were plated on chromatographic paper and then eluted.

This study was aided by grants from the Division of Research Grants and Fellowships, National Institutes of Health, U.S. Public Health Service; the Josiah Macy Jr. Foundation and the Association for the Aid of Crippled Children. The first Author is Josiah Macy Jr. Foundation Fellow.

Department of Physiology,
Yale University School of Medicine,
New Haven, Conn. (U.S.A.)

FREDERICK C. BATTAGLIA
GIACOMO MESCHIA
DONALD H. BARRON

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Received July 25th, 1960