During the experimental period, the oxygen consumption is determined manometrically in the usual way, with KOH in the center well to absorb CO₂. One advantage of this method over differential manometry for measuring tissue respiration in serum is that the time-course of respiration may be followed, and by using vessels equipped with more than one side-well. additional samples of serum may be withdrawn at intervals to follow the time-course of glycolysis.

Tests of the method show that the same figure for $Q_G^{O_2}$ is obtained by this "side-well technique," where only the serum is analyzed, as by the usual method of analyzing separate samples at the beginning and end of the experiment and including the tissue in the analysis. In the case of bone marrow, the only tissue so far studied by these methods, the Qo2 is the same in neutralized as in unaltered serum,⁴ but the $Q_G^{O_2}$ is higher in neutralized serum, apparently an effect of the absence of CO_2 . This finding may not apply to other tissues.

These methods may of course be used with any medium, but they are particularly useful when it is important to use serum, to conserve tissue and to use as few manometers as possible. The chemical method of measuring glycolysis has the advantage of being virtually specific for lactic acid, and accuracy is gained by making the initial and final analyses on one sample of tissue rather than on separate samples, as in the usual way.

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THE PRESERVATION OF OXIDIZABLE SUBSTANCES IN SOLUTION¹

CERTAIN chemical and biological preparations, notably those used for growth stimulation or containing natural sulphydryl complexes are prone to gradual oxidation and subsequent loss of potency when in solution. Shinohara² has given formulas for these inactivating oxidations and our own work Owen³ on tissue extracts are in fair agreement with his findings.

After attempting to prevent oxidation in these solutions by refrigeration, tightly capping the vials and employing antioxidants with a minimum of success we found the following procedure to be effective. The embryonic extract or solutions are prepared in the usual manner either aseptically or with permissable amounts of preservative. These are then strained or

² K. Shinohara, Jour. Biol. Chem., 109: 665-679, 1935. ³S. E. Owen, Growth, 2: 355-361, 1938.

filtered as desired. The preparation is then placed in open beakers or wide-mouthed screw cap vials and set at room temperature into a vacuum desiccator. The pump is started and sufficient vacuum applied to cause mild bubbling of the solution but little or no loss in volume by evaporation. Usually not over five minutes pumping is required to remove the dissolved air. The vacuum is then replaced with an inert gas, as carbon dioxide, slowly let in. The containers are filled to the top and the cap screwed on and tightened before removal from the desiccator. Low melting paraffin may also be used to seal the containers. All air must be excluded to insure protection from oxidation.

As an example 1:100,000 cysteine hydrochloride solutions and tissue extracts containing free sulphydryl were maintained at room temperature for over six months with no apparent loss of sulphydryl. Similar solutions not so treated usually gave a negative sulphydryl test after one to four weeks. The method of testing was that used by Owen³ employing the phospho-18-tungstic acid reagent of Folin and Marenzi.⁴

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4 O. Folin and A. D. Marenzi, Jour. Biol. Chem., 83: 109-114, 1929.

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⁴ C. O. Warren, loc. cit.

⁵ This work was done during the tenure of a Lewis Cass Ledyard, Jr., Fellowship, New York Hospital, 1940-1941.

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