This conversion of desoxycorticosterone to pregnandiol-3 (α), 20 (α) is unique in the metabolism of the steroid hormones since it is the first instance of the replacement of an hydroxyl group by a hydrogen atom. Thus the primary alcohol group at C-21 in desoxycorticosterone is reduced to the corresponding methyl group in pregnandiol-3 (α), 20 (α).

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

DEVICE FOR THE PREPARATION AND TRANSFER OF OXYGEN-FREE SOLUTIONS¹

A PROBLEM frequently encountered is the preparation of an air (oxygen)-free solution and its subsequent introduction into an experimental vessel. For example, in the course of spectrophotometric determinations involving solutions of respiratory enzymes and other proteins it is necessary to remove all traces of oxygen and then to transfer the solution to the cell



in which light absorption is to be measured. Another case is the introduction of air-free solution into a reaction cell for photochemical purposes, particularly when the analytical procedure involves spectrophotometry or colorimetry.

A very simple and widely used procedure which involves passing nitrogen through the solution is of limited usefulness. Thus in the case of protein solutions the customary method may lead to extensive foaming which results in loss of material and in denaturation of the protein. In all cases it is limited to solutions in which solvent and solutes are relatively non-volatile.

We present below a device which has been very useful in this laboratory and which seems to have general applicability. With the stopcocks A, B, C and D set as designated by "Position I," gaseous nitrogen is admitted at D and at the same time the other part of the system, including the absorption cell, is connected to a high vacuum pump for a few minutes. By gentle rotation of the flask the solution will be spread out

¹ From the George Herbert Jones Chemical Laboratory of the University of Chicago, Chicago, Illinois. and a very efficient removal of gas will take place. In order to transfer the oxygen-free solution to the cell L the stopcocks are adjusted as indicated by "Position II," where stopcock C should be operated last and rather gradually. With increasing nitrogen pressure the solution will be forced through the capillary and into the attached cell. The ground glass joint G_1 allows the detachment of the cell. The capillary stopper,² filled with solution, prevents the diffusion of air into the cell. In an alternative arrangement a stopcock is inserted in the capillary and closed at the conclusion of the transferring operation.

A modified procedure is employed in case the solution is volatile or has a volatile component. The flask is closed off by means of stopcocks B and C and its contents frozen by immersion of the flask in liquid air.³ After temperature equilibrium is attained stopcock B is turned to Position I and the system evacuated, thus removing all non-condensable gases. Then, with stopcock B closed, the flask is heated to room temperature, whereby most of the dissolved air escapes into the vacuum above the solution. The process of alternate freezing, evacuating and thawing is repeated; it was found that three such steps sufficed to remove oxygen effectively from a 12 molar hydrochloric acid solution, the concentration of acid remaining unchanged within the accuracy of the analytical method employed (0.5 per cent.).

The method as described relies to some extent on the purity of the nitrogen used. However, one may employ commercial nitrogen and avoid contamination of the solution with oxygen if a surplus of the solution is available, for the top layer of the liquid will protect the portion which is to be transferred.

The authors wish to acknowledge their indebtedness to the Rockefeller Foundation for its support of the project in which this work developed.

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