material with a sedimentation constant of 62×10^{-13}, a value essentially the same as that obtained with normal brain tissue here. The components isolated in the present work exhibited evidence of high homogeneity in the analytical ultracentrifuge. Despite the similarity of the sedimentation velocity values, the various components, including the material of chick embryo body,[4] differed constitutionally, indicating not only species but organ variation. In addition there was variation associated with age. The results suggest that further study of the normal tissue components may be of significance not only in relation to physiological processes but possibly in the investigation of non-infectious pathological processes such as neoplasms. This is supported by the results of preliminary studies now being made in this laboratory with certain brain tumors of man.

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THE CONVERSION OF DESOXYCORTICOSTERONE TO PREGNANDIOL-3 (α), 20 (α)[1, 2]

THE conversion of desoxycorticosterone acetate to pregnandiol glucuronidate in a normal man was claimed by Cuyler, Ashley and Hamblen.[4] The evidence was based on two isolations of pregnandiol glucuronidate, one m.p. 240° C and the other m.p. 261° C. The melting points of both compounds were low as compared to the melting point for pregnandiol glucuronide (268–271° C) reported by Venning and Browne.[5] No evidence for the identity of the compounds was presented in addition to these melting point determinations. In a second communication Cuyler, Hirst, Powers and Hamblen[6] were unable to reproduce their original results. In the course of a study of the metabolism of desoxycorticosterone and other adrenal cortical steroids in the chimpanzee and human, we have demonstrated the conversion of desoxycorticosterone to pregnandiol-3 (α), 20 (α) in the ovariectomized chimpanzee.

A total of three grams of desoxycorticosterone acetate[3] was administered orally over a period of 15 days. The hormone was administered once daily in doses of 200 mg. Urine was collected for the 15-day period during hormone administration and for the following three days. A total of 14.9 liters of urine was collected. Fifteen cc of concentrated hydrochloric acid and 25 cc of carbon tetrachloride were added to each 100 cc of urine. Hydrolysis and extraction were carried out simultaneously for a period of 8 hours. The carbon tetrachloride was separated and an additional quantity of fresh carbon tetrachloride added and the extraction repeated. The material soluble in carbon tetrachloride was separated into two fractions by means of 10 per cent. sodium hydroxide; one, the fraction containing the neutral compounds, and the other the fraction containing the acidic and phenolic compounds. By means of the Girard-Sandulesco ketone reagent, trimethylacethydrazide ammonium chloride and succinic acid anhydride the total neutral compounds were separated into four fractions: the ketonic-hydroxy, ketonic-nonhydroxy, nonketonic hydroxy and nonketonic-nonhydroxy fractions.

The nonketonic-hydroxy compounds were further separated with digitonin into fractions containing the soluble and insoluble digitonides, respectively. A colorimetric assay of the former fraction indicated 455 mg pregnandiol equivalent.[7] It was adsorbed on a column (10×190 mm) of Brockmann's aluminum oxide from a solution of benzene. The column was eluted with progressively increasing concentrations of ethanol in benzene ranging from pure benzene to 16 per cent. ethanol in benzene. The fraction eluted by 1 per cent. ethanol in benzene yielded a crystalline material which assayed 125 mg of pregnandiol equivalent. The material after recrystalizing three times from aqueous ethanol yielded 45 mg of a compound which melted at 228–229° C.[8] A mixture of this compound with an authentic sample of pregnandiol-3 (α), 20 (α), m.p. 229–231° C, melted at 229–230° C. The diacetate melted at 175–176° C and when mixed with pregnandiol-3 (α), 20 (α) diacetate, m.p. 174–175° C, the mixture melted at 174–175° C.

After combining the mother liquors and rechromatagraphing the crude compounds an additional quantity of 34 mg of pregnandiol-3 (α), 20 (α) was isolated. Thus the total quantity of pregnandiol-3 (α), 20 (α) recovered from 3 grams of desoxycorticosterone acetate totaled 79 mg, indicating a conversion of 3 per cent. In a second experiment when 1.2 grams of desoxycorticosterone acetate was administered to the same ovariectomized chimpanzee, 12 mg of pregnandiol-3 (α), 20 (α) were recovered in the urine. This represented a conversion of 2 per cent.

[1] Supported in part by the Josiah Macy, Jr. Foundation. Accepted for publication in SCIENCE, November 14, 1942.

[2] From the Brush Foundation and Department of Biochemistry, Western Reserve University School of Medicine and the Department of Medicine, Lakeside Hospital, Cleveland, Ohio.

[3] The desoxycorticosterone acetate was kindly supplied by the Ciba Pharmaceutical Products Inc. under the trade name of Percorten.

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[8] Melting points are uncorrected and were determined by means of the Fischer-Johns apparatus.

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 SOLUTIONS[1]

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

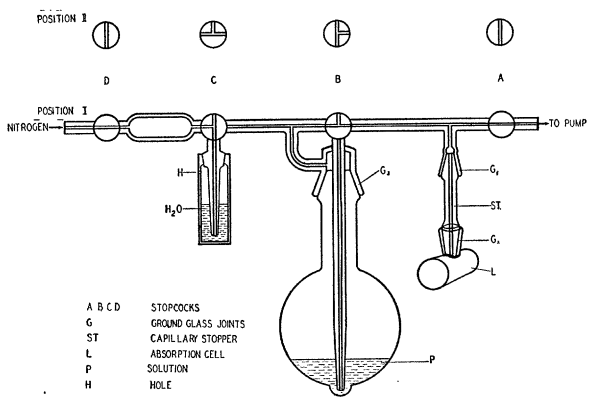

FIG. 1.

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 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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