SPECIAL ARTICLES

MACROMOLECULAR COMPONENTS OF NORMAL EMBRYONIC AND ADULT BRAIN TISSUE[1]

ULTRACENTRIFUGAL studies on plant[2] and animal tissues[3] have resulted in the demonstration in healthy organisms of constituents with high sedimentation constants. Recently, a material with a sedimentation constant of $S_{20} = 73.8 \times 10^{-13}$ has been obtained from extracts of chick embryo body tissue, and analyses have shown it to be a lipoprotein complex containing nucleic acid of the ribose type.[4] The frequency with which these components have been encountered and the high concentrations of them occasionally observed indicate not only a wide distribution but also a role of importance in the physiological processes of healthy tissues. In the work described here the question of distribution has been investigated further in studies on brain tissues from man and certain animals.

The studies were made on the whole brain tissue of man, the rabbit and the chick. Both embryonic and adult tissues were examined by procedures similar to those used in isolation of the normal component of the chick embryo body.[4] Fresh tissue was chilled and quickly ground either with sand in a Ten Broeck grinder or in the Waring Blendor. The mince was extracted in 20 per cent. suspension in distilled water for 48 hours at 5 to 8° C. After clarification with the angle centrifuge, the extract was filtered with celite and subjected to 1 or 2 cycles of ultracentrifugation at 67,000 g for 30 minutes. The resulting pellets were dissolved and stored in distilled water.

Two human embryos were studied. The brain from one of these, crown-rump measurement 16.5 cm, weighed 41 gm. From the extract of the cerebral tissue clear gel-like pellets were obtained representing 0.17 mg of nitrogen per gram of initial brain tissue. Solutions of the pellets in concentrations of 0.45 to 0.55 mg N per cc examined in the analytical ultracentrifuge showed sharp sedimenting boundaries indicative of considerable homogeneity. No light-absorbing material was seen above the boundary but beneath it there was present some material which sedimented more quickly than the component giving the sharp boundary. The sedimentation velocity in water was 70.8×10^{-13}. A similar component seen in the second embryo of about the same age gave a sedimentation velocity of 67.5×10^{-13}. Partial elementary analysis showed a composition of N, 12.7; C, 51.4; and P, 2.81 per cent. The density by pyknometer measurement was 1.42. The Millon and biuret tests were positive, and the glyoxylic acid test was weakly positive after 20 minutes. Bial's test following hydrolysis was negative. The Feulgen test was positive and so, too, was the diphenylamine reaction. These results indicate a material essentially protein in nature, possibly associated with a small quantity of fat and containing nucleic acid of the desoxypentose type.

Similar experiments with 4 batches of brain from chick embryos of 14 to 20 days incubation resulted in the purification of a component showing very sharp boundaries with a sedimentation velocity in water of 67.5×10^{-13}. The sedimentation constant in 0.095 M borate buffer, pH 7.2, was 75.2×10^{-13}. The constitution of this component was wholly different from that of human embryo brain but somewhat similar, though not identical, to the chick embryo body component.[4] The carbon content was 50.9, nitrogen 11.3 and phosphorus 2.9 per cent. Bial's test in this instance was strongly positive and the diphenylamine test dubiously positive, indicating a further resemblance of this brain component to that of the body tissue in the presence of ribonucleic acid. The yield was 0.1 mg N per gram of brain.

Experiments with the brain of rabbit embryos yielded a constituent giving good sedimentation diagrams and a sedimentation velocity in water of 68.9×10^{-13}.

Examinations of adult brain from man, chick and rabbit resulted in all cases in preparations showing very diffuse sedimentation diagrams under the same ultracentrifugal conditions giving sharp boundaries with embryo material. The sedimentation velocity of the adult brain material was of the same magnitude as that from embryo tissue. This finding indicated great heterogeneity with respect to particle size. Another striking difference between the embryo and adult was the yield of product which was approximately 5 times greater for the embryo than for the adult. Electron micrographs of the materials from human and chick embryo brain showed rounded images approximately 18 mμ in diameter for both. The images appeared uniform in size and no internal structure was visible.

Thus far the components of normal tissues have been encountered chiefly as complicating factors in the purification of viruses such as those of certain plant diseases and the agent of equine encephalomyelitis.[5] Recent studies[6] on poliomyelitis have revealed in diseased cord and medulla of monkeys a

[1] This work was aided by the Dorothy Beard Research Fund and by a grant from Lederle Laboratories, Inc., Pearl River, N. Y.

[2] W. C. Price and R. W. G. Wyckoff, *Phytopathology*, 29: 83, 1939.

[3] R. W. Glaser and R. W. G. Wyckoff, *Proc. Soc. Exp. Biol. and Med.*, 37: 503, 1937.

[4] A. R. Taylor, D. G. Sharp, D. Beard and J. W. Beard, *Jour. Infect. Dis.*, 71: 110, 1942.

[5] A. R. Taylor, D. G. Sharp, D. Beard and J. W. Beard, *Jour. Infect. Dis.*, in press.

[6] H. S. Loring and C. E. Schwerdt, *Jour. Exp. Med.*, 75: 395, 1942.

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.

[4] W. K. Cuyler, C. Ashley and E. C. Hamblen, *Endocrinology*, 27: 177, 1940.

[5] E. M. Venning and J. S. L. Browne, *Proc. Soc. Exp. Biol. and Med.*, 34: 792, 1936.

[6] W. K. Cuyler, D. V. Hirst, J. M. Powers and E. C. Hamblen, *Jour. Clin. Endocrinology*, 2: 373, 1942.

[7] N. B. Talbot, R. A. Berman, E. A. MacLocklan and J. K. Wolfe, *Jour. Clin. Endocrinology*, 1: 668, 1941.

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