Proceedings
of the
Society
for
Experimental Biology and Medicine

VOL. 78 OCTOBER, 1951 No. 1

Observations on a Rapidly Migrating Electrophoretic Component of Cerebrospinal Fluid.*† (18954)

ALBERT A. FISK, ALFRED CHANUTIN, AND WALTER O. KLINGMAN.
(Introduced by C. L. Gemmill.)

From the Biochemical Laboratory and the Department of Psychiatry and Neurology, University of Virginia Medical School, Charlottesville, Va.

Cerebrospinal fluid has been shown to contain an electrophoretic component with a migration rate faster than albumin in 4 of 62 cases studied by Kabat *et al.*(1) and by Scheid and Scheid(2). No evidence was presented by these investigators which would associate a specific disease with this fast component. Evidence is presented in this paper that a rapidly migrating protein is a normal constituent of cerebrospinal fluid.

Methods. Cerebrospinal fluids were obtained from patients undergoing pneumoencephalograms for diagnosis. Fluids collected at the University Hospital were centrifuged immediately and stored in the cold room. Samples collected at the Veterans Hospitals in Richmond and Roanoke were stored in a refrigerator until they were transported to this laboratory in an iced thermos jug. It was determined that storage does not affect the spinal fluid proteins as determined electrophoretically. Fluids showing visible evidence of blood were discarded.

The volumes of the spinal fluid varied between 60 and 200 ml. Each sample was concentrated in the cold room at 4° to a volume of 2-4 ml by positive nitrogen pressure dialysis through collodion sacks(3). The average spinal fluid was concentrated in about 24 hours. This material was dialyzed against a potassium phosphate buffer at pH 7.9 and ionic strength of 0.05.

The electrophoretic technic of Scheid and Scheid(2) was used in a Klett Tiselius apparatus. The lower descending and the horizontal limbs of an 11 ml separation cell were filled with dialyzed plasma. The lower ascending limb was filled with concentrated spinal fluid and the system was filled with the phosphate buffer. The boundary between the spinal fluid and the buffer was sharpened by slowly syphoning buffer through a glass capillary with a right angle tip attached to polyethylene tubing(4,5). Electrophoresis proceeded for one hour at a potential gradient

* This investigation was supported by Research Contract V1001M-1074, Veterans Administration.

† The authors are indebted to the staff members of the University of Virginia Hospital and of the Veterans Hospitals in Richmond and Roanoke for collecting spinal fluids and to Titus Pankey for technical assistance.

1. Kabat, E. A., Moore, D. H., and Landow, H., *J. Clin. Invest.*, 1942, v21, 571.

2. Scheid, K. F., and Scheid, L., *Arch. Psychiat. Nervenkrankh.*, 1944, v117, 219, 641.

3. Personal communication from Dr. J. L. Oncley, Harvard University.

4. Longsworth, L. G., *J. Am. Chem. Soc.*, 1945, v67, 1109.

5. Kahn, D. S. and Polson, A., *J. Phys. Coll. Chem.*, 1947, v51, 816.

of 3.65 volts/cm. Records were obtained by Longsworth's scanning method(6). Electrophoresis was continued at a potential gradient of 5.48 volts/cm. The albumin boundary was kept stationary by continuous compensation achieved by withdrawing buffer from the upper descending limb through narrow polyethylene tubing. The rate of flow was controlled by adjusting the level of the outflow. After sufficient electrophoresis, the fast component was collected from the upper two-thirds of the ascending limb. These separated samples were immediately frozen and were subsequently thawed, pooled and concentrated by pressure dialysis.

The sedimentation rates were determined in a Model E Spinco ultracentrifuge.

Results. A clearly resolved component of anodic mobility greater than albumin is observed in each of the 65 cerebrospinal fluids studied. This protein, designated as component X, has a mean mobility of 11.8 $\times$ 10^{-5} cm^2 $volt^{-1}$ $sec.^{-1}$, indicating a migration rate 1.3 times faster than albumin. In 9 cases a small component of anodic mobility greater than component X was resolved. This protein, designated as component X_1, has a mean mobility of 14.0 $\times$ 10^{-5} cm^2 $volt^{-1}$ $sec.^{-1}$. Preliminary analyses indicates that component X is resolvable between pH 4.0 and 7.9 and has an isoelectric point between pH 4.0 and 4.5. An electrophoretic pattern showing both components is presented in Fig. 1.

The mean relative concentration of component X in dilute phosphate buffer is approximately 9% of the total protein with individual values varying between 3 and 17%. The true values for this component are probably less since boundary anomalies are appreciable in dilute phosphate buffer. No differences in the concentrations of component X are observed in spinal fluids from patients with schizophrenia, brain tumor or epilepsy. The mean relative concentration of component X_1 is about 1% of the total protein; its presence could not be associated with any particular disease.

The separated fast components of 30 spinal fluids were pooled and concentrated by pressure dialysis. The electrophoretic pattern of this concentrated fraction (Fig. 2) shows the presence of 2 main components. The larger component has the mobility of component X and represents 63% of the total area. The asymmetry of the boundary at the leading edge is presumably due to component X_1. The remaining component has the mobility of albumin. Ultracentrifuge analysis of the concentrated pooled fractions indicates that over 95% of the protein sediments as a homogeneous component with the sedimentation

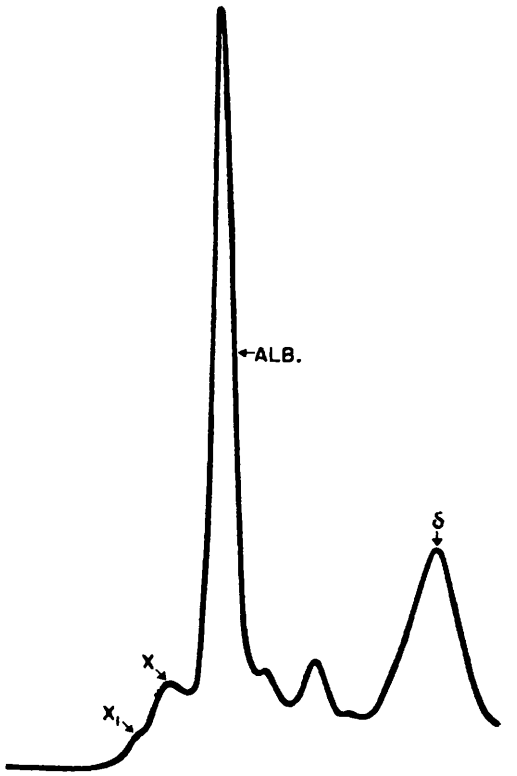

FIG. 1. Electrophoretic pattern of concentrated cerebrospinal fluid.

FIG. 2. Electrophoretic pattern of concentrated rapidly migrating components of cerebrospinal fluid.

6. Longsworth, L. G., *Ann. New York Acad. Sc.*, 1939, v39, 187.

rate of albumin. The remaining protein sedimented 2.2 times more rapidly than the main component.

Discussion. A rapidly migrating component is readily observed in the electrophoretic patterns of concentrated spinal fluids equilibrated with a phosphate buffer (pH 7.5, $\Gamma/2$ .05) or a veronal buffer (pH 8.6, $\Gamma/2$ 0.1). Kabat *et al.*(1) using a phosphate-sodium chloride buffer mixture at pH 7.4 and an ionic strength of about 0.2, recorded the presence of a fast component in 3 of 40 spinal fluids. Scheid and Scheid(2) studied spinal fluids which were not sufficiently concentrated and observed the fast component in 1 of 22 cases. These studies emphasize the necessity of using concentrated fluids and suitable buffer mixtures if the rapidly migrating components of spinal fluid are to be observed or separated electrophoretically.

Component X has a greater anodic mobility than albumin over a pH range varying from 7.9 to 4.0. In contrast, rapidly migrating components observed in plasma do not behave similarly over this pH range(7). Furthermore, plasma adjusted to the protein concentration of concentrated spinal fluid and equilibrated against dilute phosphate buffer does not reveal the presence of component X. Under these conditions the albumin boundary of plasma spreads appreciably and may prevent the detection of small amounts of the fast component. From the available data, it appears justifiable to assume that components X and X_1 originate in the nervous system and are proteins typical for cerebrospinal fluid.

Summary. Electrophoretic studies of 65 cerebrospinal fluids showed the presence of a rapidly migrating protein in each case. This component migrated about 1.3 times faster than albumin and sedimented at the same rate as albumin in the ultracentrifuge. In 9 cases, 2 rapidly migrating components were observed. Under the experimental conditions used, the fast components comprise about 9% of the total spinal fluid proteins.

7. Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M., *J. Clin. Invest.*, 1948, v27, 609.

Received July 30, 1951. P.S.E.B.M., 1951, 78.

Hydrolysis of Conjugates of Neutral Ketosteroids. (18955)

HENRY J. BUEHLER,* PHILIP A. KATZMAN, AND EDWARD A. DOISY.
From the Department of Biochemistry, Saint Louis University School of Medicine, Saint Louis, Missouri.

The study of the metabolism of steroids is, in part at least, dependent upon the determination of the quantities and types of steroids excreted in the urine. Important information could also be obtained if it were possible to estimate the individual steroid conjugates so excreted.

The usual method of hydrolyzing the steroid conjugates by refluxing strongly acidified urine has been shown to cause alterations of at least some of the steroids(1-9). Furthermore, such treatment would be expected to hydrolyze all of the conjugates to a greater

* Atomic Energy Commission Predoctoral Fellow in the Biological Sciences.

1. Butenandt, A., and Dannenbaum, H., *Z. Physiol. Chem.*, 1934, v229, 192.

2. Astwood, E. B., and Jones, G. E. S., *J. Biol. Chem.*, 1941, v137, 397.

3. Pincus, G., and Pearlman, W. H., *Endocrinology*, 1941, v29, 413.

4. Talbot, N. B., Ryan, J., and Wolfe, J. K., *J. Biol. Chem.*, 1943, v148, 593.

5. Venning, E. H., Hoffman, M. M., and Browne, J. S. L., *Endocrinology*, 1944, v35, 49.

6. Lieberman, S., Dobriner, K., Hill, B. R., Fieser, L. F., and Rhoads, C. P., *J. Biol. Chem.*, 1948, v172, 263.

7. Stimmel, B. F., *J. Biol. Chem.*, 1949, v178, 217.

8. Bitman, J., and Cohen, S. L., *J. Biol. Chem.*, 1949, v179, 455.

9. Dingemanse, E., Huisin't Veld, L. G., and Hartogh-Katz, S. L., *Nature*, 1948, v162, 492.