at the time ECHO 28 was prevalent.

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Separation of Brain Proteins by Starch Gel Electrophoresis.* (26750)

JOSEPH BERNSOHN, KEVIN D. BARRON AND ADELINE R. HESS
(Introduced by E. Kaplan)
Neuropsychiatric Research Laboratory, Veterans Administration Hospital, Hines, and Northwestern University Medical School, Chicago, Ill.

The complex nature of the mixture of proteins occurring in most tissues has rendered their identification and characterization exceedingly difficult. Analysis by conventional Tiselius technics and paper electrophoresis procedures have not yielded definitive results. The use of agar gel and starch gel (1,2) as supporting media for electrophoresis studies provides some superiority over paper in that more fractions have been visualized, and both have been successfully employed for studies of serum proteins. However, the applicability of these procedures to the study of tissue proteins has been limited. Voskobolnokov(3) reported on the separation of liver proteins using agar gel and was able to identify 13 separate moieties. Karcher, van Sande and Lowenthal(4) have reported on the separation of central nervous system proteins on agar gel in various pathological conditions. They characterized the proteins in a manner analagous to serum proteins and demonstrated 7 protein bands in normal white matter and 8 in normal brain stem, using a densitometer to quantitate their material. Eariler studies using paper electrophoresis(5) had inadequately resolved brain proteins although Palladin and Poliakova(6) reported the presence of 7-8 fractions in the central nervous system of the cat using this technic.

In this study, starch gel was used as the supporting medium, and the vertical electrophoresis procedure(7) was adopted. This yielded a satisfactory separation of the protein moiety, which appeared superior to that reported by the other methods mentioned. While this work was in progress Bailey and Heald(8) reported on use of horizontal starch-gel electrophoresis for separation of central nervous system proteins. They achieved some satisfactory delineation of the proteins.

Methods. Adult rats were sacrificed by fracturing the spinal column with the aid of a heavy metal bar, and the brain was extir-

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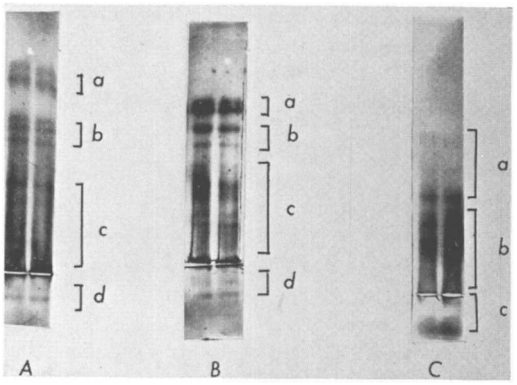

FIG. 1. Starch-gel electrophoresis of brain proteins. A. Rat brain, unperfused. B. Rat brain, perfused. C. Human brain, white matter.

pated immediately. This procedure was performed usually in less than one minute. The brain was placed into one volume (g/ml) of either cold distilled H_2O or isotonic NaCl and homogenized. It was found that both extractives yielded similar results while butanol extraction gave inferior separations. The homogenates were frozen and thawed 5 times and then centrifuged at 2°-4°C at 20,000 × g for 60 minutes. Centrifugation at lower speeds (*viz.* 3500 × g) yielded preparations which gave poor results. Fifty lambda of the homogenate was then placed in the slit of the block. Rat blood was obtained by exsanguination on a different group of animals and the serum run directly.

Human material was obtained from post-mortem specimens, usually within 6 hours after death and preparation of the extract was the same as described above.

The starch was obtained from Connaught Medical Research Laboratories, and preparation of the gel as well as separation were carried out as described by Smithies(7).

After some trials a .025M borate buffer at a pH 8.9 was employed for preparation of the starch gel and a voltage gradient of 4.5 volts/cm was applied across the cell. The sample was placed in the slit, then the block was covered with saran wrap after the slit had been overlaid with a mixture of vaseline and mineral oil. The apparatus was then placed in a 13°C incubator and run for 14-18 hours. After fractionation was completed, the starch block was placed on the table and cut in half in a horizontal plane and protein was visualized by staining with Amido black B and washed as described by Pert *et al.*(8).

Results. Rat brain. Separations were performed on white and grey matter and corpus striatum, but because no qualitative differences could be discerned between the homogenates of the 3 structures, the results obtained on whole brain are shown in Fig. 1A & 1B as representative of the type of fractionation achieved. Perfused and unperfused brain are depicted in the unretouched photograph. The two types of preparation have in common the appearance of 2 leading bands, of albumin-like migration characteristics (a). This was demonstrated by running serum albumin simultaneously, and noting that its mobility corresponded to that of the slower of the 2 bands. Both perfused and unperfused brain have in common the appearance of 2 bands migrating toward the cathode (d), presumably γ-globulin-type proteins, and both preparations contain 4-5 bands on the anode side nearest the origin (c). The effect of perfusing the brain is shown in what is presumably the alpha-globulin region where 3 close-appearing bands are seen in the unperfused preparation, but one of these bands is absent in the perfused tissue (b). Whether this represents hemoglobin or is related to the perfusion technic is undetermined. Thus 11-13 bands can be separated and visualized in rat brain by this procedure.

Human white matter. The fractionation achieved with human white matter is shown in Fig. 1C. There are 3 bands migrating toward the cathode (c), then 7-8 light-staining bands appearing in a position analagous to serum albumin and alpha-globulin (a), and 4 bands, characterized by a heavy staining band at about the center of the electrophorogram (b). Whether this represents blood contamination is undetermined. In all, the white matter seems to separate into 14-15 bands compared with the 11-12 obtained above in rat brain. It is difficult to ascertain what contribution blood may make to the proteins visualized since perfusion of this material was not attempted.

Discussion. It is apparent that human white matter contains only a small percent-

age of proteins of albumin characteristics in terms of mobility, and differs markedly from blood serum in this respect. This paucity of albumin in white matter has also been reported in paper electrophoresis studies(9,10), but the results with agar(4) demonstrated appreciable amounts of this type of protein. That these observed differences can be attributed to the two types of technics employed seems improbable, and our data tend to support the results obtained on paper. The rat brain shows considerably more albumin-type protein, as well as a definite specie difference in the number as well as mobility of other protein fractions. Results obtained by other workers with various species would indicate that considerable variations in type and concentration of various brain proteins exist between species. While this might be expected on the basis of an analogy with blood serum, it would seem that less variation might occur in brain, because of the basic neural function of the organ, and its relative independence of normal external conditions to maintain its structural integrity. Whether these differences may ultimately be correlated with some of the higher cerebral function of human brain is a moot point.

No attempt was made to quantitate the data as it was felt that conventional dissection and elution technics presented a problem which is not now too readily soluble. First, the thickness of the gel cannot be controlled during the cutting procedure so that assay of the protein by measurement of 280 mμ absorbing material did not seem feasible, and secondly, determination of dye intensity by extraction of the protein from the starch did not seem satisfactory because of the varying degrees of background staining along the gel. Measurement of color intensity either by a densitometer or reflectometer has not been attempted as it was felt that some of the same criticisms applied. It would seem that characterization of the proteins in brain would be of greater significance than quantitation. Our efforts are being directed to the former problem.

Summary. Starch-gel electrophoresis has been employed to separate the proteins in human and rat brain. This technic yielded results superior to those attained by paper or agar-gel electrophoresis. Separation of water-soluble proteins on starch-gel yielded 11-12 discrete protein bands in rat brain and 14-15 bands in human white matter. Human white matter proteins are characterized by low concentration of albumin-type proteins.

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