significant comparisons can be made, however, with the action of LSD upon other central pathways. Purpura(7) has postulated that those cortical tracts which have predominantly axosomatic synaptic connections are facilitated by LSD. Since the connections of muscle afferents with spinal motoneurones are principally axosomatic(8) facilitation of the monosynaptic spike is to be expected.

Also pertinent is the concept that certain cortical association tracts(9), midbrain reticular pathways(10) and spinal reflexes(11) contain adrenergic links. The present study shows that LSD modifies spinal reflexes in a manner analogous to that of epinephrine(12). Similar evidence has been presented in experiments on the transcallosal preparation(9). LSD may, therefore, interact with proposed adrenaline-like transmitters.

Summary. The amplitude of the monosynaptic reflexes recorded from the ventral roots of unanaesthetized spinal cats was found to be increased after i.v. injection of LSD in doses greater than 200 μg/kg. Polysynaptic activity was depressed coincidentally with facilitation of the monosynaptic spike.

1. Weidmann, H., Cerletti, A., *Helv. Phys. Acta,* 1957, v15, 376. Little, K. D., DiStefano, V., Leary, D. E., *J. Pharm. Exp. Therap.,* 1957, v119, 161; DiStefano, V., Leary, D. E., *Fed. Proc.,* 1958, v17, 364.

2. Isbell, H., Belleville, R. E., Fraser, H. F., Wikler, A., Logan, C. R., *Arch. Neurol. Psychiat.,* 1956, v76, 468.

3. Lloyd, D. P. C., *J. Neurophysiol.,* 1943, v6, 111.

4. Geiger, L. E., Cervoni, P., Bertino, J., Monteleone, F., *J. Pharm. Exp. Therap.,* 1958, v123, 164.

5. Rothlin, E., *Ann. N. Y. Acad. Sci.,* 1957, v66, 668.

6. Elder, J. T., Gogerty, J. H., Dille, J. M., *Fed. Proc.,* 1957, v16, 293.

7. Purpura, D. P., *Arch. Neurol. Psychiat.,* 1956, v75, 132.

8. Lorente de No', R., *J. Neurophysiol.,* 1938, v1, 195.

9. Marrazzi, A. S., Hart, E. R., *Science,* 1955, v121, 365.

10. Dell, P., Bonvallet, M., Hugelin, A., *EEG and Clin. Neurophysiol.,* 1954, v6, 599.

11. Skoglund, C. R., *Cold Spring Harbor Symp. on Quant. Biol.,* 1952, v17, 223.

12. Wilson, V. J., *Am. J. Physiol.,* 1956, v186, 491.

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Zone Electrophoresis of Cerebrospinal Fluid Proteins in Starch Gel.* (24288)

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The observation that total protein in cerebrospinal fluid may increase substantially in many diseases of the nervous system has stimulated considerable investigation of the protein fractions with new methods of protein separation. It has been hoped that when specific changes were found, they would be of diagnostic value and would shed some light on the large number of neurologic diseases of unknown cause. The exceptional resolution of serum protein fractions by electrophoresis in starch gel described by Smithies (1), and our(2) experience with this method, whereby we can obtain 15 to 20 protein fractions in normal sera, suggested that comparable fractionation might be gained by applying this technic to cerebrospinal fluid protein.

Methods and materials. Specimens of spinal fluid and serum were obtained from patients in the hospital. Most of these patients suffered from disease of the nervous system; only a few could be considered normal subjects. After determining concentration of protein of spinal fluid by the sulpho-

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salicylic acid turbidity method(3), the specimen was concentrated to give an approximate final concentration of 1 g% protein. Concurrently a serum specimen was diluted with 0.9% sodium chloride to 50 or 100 times its original volume and concentrated by the same method as the spinal fluid. The principal method of concentration was dialysis of the specimen in cellophane bags against 35% solution of Polyvinylpyrrolidone (PVP).† Usually 10 ml of cerebrospinal fluid were concentrated to 0.2 ml. To assure recovery of the necessary volume of concentrate, a glass container of appropriate size was incorporated into the bottom of the bag. Containers of 0.2 ml to 2 ml were designed to allow concentration from 1-50 to 1-5. The appropriate amount of granular starch was placed in the container so that after concentration, the specimen was ready for application in the starch gel electrophoresis run. Care was taken to remove any air bubbles from the glass container prior to tying the top of bag. A small weight was attached to bottom of container to keep the bag in the vertical position. This method allows predetermination of volume of final concentrate and minimizes the danger of losing the specimen.

When specimens contain less than 25 mg% of protein, it is necessary to concentrate the material as much as 100 times, and under these circumstances we found lyophilization more satisfactory because of the small volume of final concentrate. Prior dialysis is then necessary to eliminate excess electrolyte so that final electrolyte concentration is approximately the same as that existing before concentration. Lyophilization, however, has the disadvantage of altering lipoproteins and is not necessary with most pathologic specimens, because the protein concentration is usually elevated.

Electrophoresis conditions. Preparation of soluble starch and starch gel was similar to that described by Smithies(1). Details of method of preparing soluble starch for quantitative studies of electrophoresis of serum proteins in starch gel are being published(2). For spinal fluid studies we used a starch which is not hydrolyzed‡ as long as the starch used for serum studies. These investigations utilized Idaho starch, Batch #584, manufactured by Magic Valley Processing Co., Twin Falls, Idaho.§ The gel was prepared by heating 13.5 g of soluble starch with 100 cc of 0.030 M borate buffer pH 9.05. Because of the small amount of protein in the sample, we employed a plastic form with cross-sectional area approximately half that used for routine serum work. It measured 7 mm by 225 mm and was 6 mm deep. Optimal running time was 6 hours at 2 milliamperes/gel strip at 14°C. After completion of electrophoresis, the gel strip was removed from the plastic mold and cut in half longitudinally. One-half was stained with naphthalene black B 200 to visualize the proteins, the other half was stained with Oil Red O to show lipoproteins. The lipoprotein stain was prepared by making a saturated solution of Oil Red O in methanol : acetic acid : water, 60 : 10 : 30. The gel strips were left in this solution for 48 hours with 2 changes of fresh stain. They were then washed with continuous flow of tap water until background staining was reduced to a minimum. Permanent records were made through photography and with a photoelectric reflectometer designed for this purpose.‖ With this instrument a continuous scan of each strip is carried out and a graph of the stained proteins is obtained (Fig. 1,2).

Results. The serum protein patterns were similar to those previously reported(1), and for convenience we have used the same nomenclature when possible. The orientation of fractions in cerebrospinal fluids was similar to that in serum of the same patient (Fig. 1); however, differences were also obvious. The faster of the 2 pre-albumins was always considerably increased over that found in

† Generously supplied by Abbott Laboratories, Chicago, Ill.

‡ Soluble starch for serum studies is prepared by mixing 300 g of preheated starch and 600 cc acetone HCl (100:1) and let stand for 70 min. at 36.4°C. For cerebrospinal fluid studies contact time is reduced 10%.

§ Obtained from Morningstar-Nicol, Inc., N.Y.C.

‖ Generously supplied by Biophysics Dept. of Airborne Instrument Co., Mineola, L.I., N. Y.

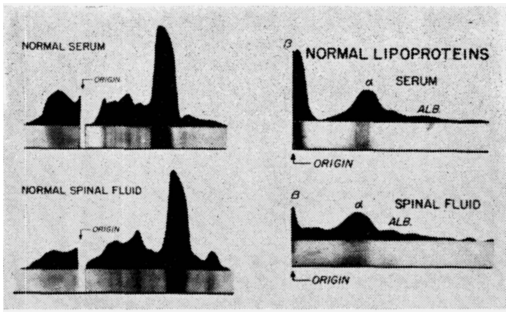

FIG. 1. An example of normal spinal fluid and serum electrophoresis patterns. Strips on left, stained with naphthalene black B, illustrate differences in protein distribution between the 2 fluids. Strips on right were stained with Oil Red O for lipoproteins; their reflectometer curves were made at higher magnification.

serum, as were the bands in the beta area. Concentration of gamma globulin, and to a lesser extent haptoglobins, was less in the spinal fluid. The most marked reduction was in slow alpha-2, the major high molecular weight fraction of alpha-2 globulins. Approximately 25% of spinal fluid samples examined showed a narrow, sharp band which migrated toward the cathode but was clearly separated from the gamma fraction (Fig. 2). This "fast gamma" fraction has not been seen in corresponding sera, has not been reported to exist in sera by other workers who have employed the same technic of electrophoresis, and has not been found in normal spinal fluid examined by us. It has been noted in such diseases as multiple sclerosis, primary lateral sclerosis, thrombosis of posterior inferior cerebellar artery, and seizure disorder of unknown origin, and was noted in one patient in 2 successive samples drawn 2 months apart. A number of abnormal spinal fluid protein patterns are illustrated in Fig. 2. It is not intended to suggest that these protein patterns are characteristic of the disease, but rather to demonstrate types of abnormalities which may be encountered. The pattern in cerebrospinal fluid specimen from a patient with bacterial meningitis shows poor separation of proteins in the alpha-beta region. In addition, 4 pre-albumin fractions are noted. Since only 2 pre-albumin fractions have been noted in any other condition, this latter finding may be of significance. The multiple sclerosis specimen illustrates changes in gamma as well as increases in post-albumin globulins. In the specimen from patient with Guillain-Barre Syndrome, a striking increase in gamma globulin is noted.

Lipoproteins appeared in 2 areas on the gel strip (Fig. 1). One, close to the origin, consisted of a wide zone with dense front migrating toward the anode. The other migrated in the post-albumin region. By experiments with isolated lipoproteins, the fraction close to the origin was identified as beta lipoprotein (low density lipoprotein floating in medium of density of 1.063); the fraction close to the albumin corresponded to alpha lipoprotein (high density lipoprotein which sediments in bottom of tube in medium of 1.063 density). Changes in alpha and beta lipoprotein fractions in spinal fluid generally followed alterations noted in the serum. The only consistent observation, however, is that the beta lipoprotein in spinal fluid is relatively decreased compared to that found in serum. The lipoprotein patterns of a normal serum and cerebrospinal fluid are illustrated in Fig. 1.

Discussion. When free electrophoresis(4, 5,6,7) and paper electrophoresis(8,9,10) were employed in separation of cerebrospinal fluid protein, albumin, alpha-1, alpha-2-, beta-, and gamma globulins were regularly noted. When concentration is adequate, 2 fractions, X and X-1, may be seen to migrate in front of albumin(5,6). In addition, some investigators

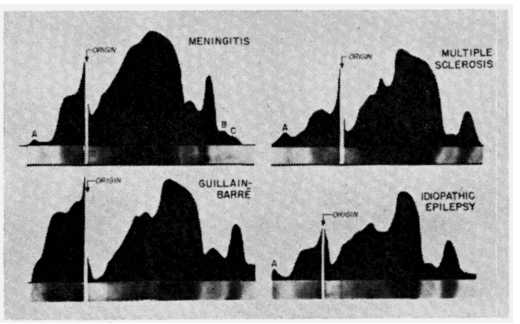

FIG. 2. Examples of abnormalities of spinal fluid protein distribution in 4 disease states. The "fast gamma" (see *A* above) can be seen in specimen of bacterial meningitis, multiple sclerosis, and idiopathic epilepsy migrating to left and separate from gamma. The meningitis shows 2 additional prealbumins (see *B* and *C* above).

have noted other fractions which migrate in the beta-gamma region, and have designated them as *tau*(6,8,11), *phi*(7,12), and gamma-2 (13). Success in demonstrating the X, X-1, tau, phi, and gamma-2 fractions has been variable, depending upon method employed. There is general agreement, however, that there are few, if any, characteristic changes in protein pattern in cerebrospinal fluid in disease states.

When specific changes are demonstrated, we might expect that they will be revealed by a technic which separates the proteins into individual fractions rather than into groups. Although neither our technic nor any other technic reported provides for such precise fractionation, the method herein described may be a step in this direction.

In spinal fluid electrophoresis it is difficult to utilize the full potential of this method of protein separation because the total sample may be as little as 1 mg. If electrophoresis were carried out in the manner which would produce optimal fraction separation in serum, many low concentration fractions in spinal fluid would not be demonstrable. A compromise between fraction identification and fraction separation must be made. To accomplish this we have used a somewhat underhydrolyzed starch, a 6-hour rather than a 16-hour running time, and a plastic form with a cross-sectional area somewhat less than half of that used for routine serum work. As a result of changing the technic, we can regularly identify only 10 to 12 fractions in serum or spinal fluid rather than 15 to 20 obtainable with the larger protein application available with serum under optimal electrophoresis conditions. We do not know how critical this limitation will be in detecting specificity of protein change in disease states.

An additional problem in determining specificity of protein distribution in disease states, is the desirability of quantitation. With paper electrophoresis we have shown that as total protein increases, the percent of each fraction increases at different rates (unpublished). Without quantitation, this factor cannot be taken into account and only large protein alterations of fractions normally present will be distinguishable from normal variation. As used at present, however, the starch gel method is useful for exploring occurrence of qualitative changes in protein in disease states.

Changes of lipoprotein patterns in cerebrospinal fluid have been noted by other investigators(10,14,15). In starch gel electrophoresis, lipoproteins migrate in areas where no major protein components are present (Fig. 1). This is an advantage since it aids in differentiating lipoproteins from high concentration protein fractions which may have some yellow color unstained and may adsorb small amounts of Oil Red O.

With the method described, no fractions have been observed in normal spinal fluid which were not present in the serum of the same patient. However, in abnormal spinal fluid, fractions have been noted which were not detected in the sera. Starch gel electrophoresis does confirm the observation from other methods that there are significant differences in relative concentration of the fractions in the 2 fluids. The low concentration of slow alpha-2 suggests that large molecular size is a factor in determining which protein can cross the blood-brain barrier. This relationship, however, is a complex one.

Summary. 1. A method for separation of cerebrospinal fluid proteins by electrophoresis in starch gel is presented, and its advantages and limitations are discussed. 2. In normal spinal fluid 10 to 12 protein fractions are observed. These correspond to serum proteins in the same individual but are present in different relative concentrations. In a number of cerebrospinal fluids from patients with neurologic diseases, considerable alterations of protein patterns were noted. Some examples of the type of abnormalities which may be encountered are illustrated. 3. In some of these pathologic specimens protein fractions were found, which we have not yet seen in serum or in normal spinal fluid. These have been designated as "fast gamma" and third and fourth prealbumins. 4. A method for concurrently staining lipoproteins in cerebrospinal fluid and serum has been described.

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1. Smithies, O., *Biochem. J.*, 1955, v61, 629.
2. Pert, J. H., Sleisenger, M. H., Woods, K. R., Engle, R. L., *Clin. Research Proc.*, 1957, v5, 156.
3. Looney, J. M., Walsch, A. I., *J. Biol. Chem.*, 1939, v127, 117.
4. Kabat, E. A., Moore, D. H., Landow, H., *J. Clin. Invest.*, 1942, v21, 571.
5. Fisk, A. A., Chanutin, A., Klingman, W. O., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 1.
6. Hoch, H., Chanutin, A., *ibid.*, 1952, v81, 628.
7. Tanaka, Z., *Tohoku J. Exp. Med.*, 1956, v63, 245.
8. Bucher, Th., Matzelt, D., Pette, D., *Klin. Wschr.*, 1952, v30, 325.
9. Delank, H. W., *Deutsche Z. f Nervenheilkunde*, 1955-1956, v174, 429.
10. Bauer, H., *ibid.*, 1956-1957, v175, 354.
11. Pluckthun, H., Matthes, A., *Z. f Kinderheilkunde*, 1953, v72, 521.
12. Ewerbeck, H., *Klin. Wschr.*, 1950, v28, 692.
13. Delank, H. W., Machetanz, E., *Deutsche Z. f Nervenheilkunde*, 1955-1956, v174, 189.
14. Baudouin, A., Lewis, J., Hillion, P., *Compt. rend. Soc. Biol.*, 1953, v147, 1036.
15. Smith, J. K., Gerstl, B., Davis, W. E., Orth, D. L., *Arch. Neurol. Psychiat.*, 1956, v76, 608.

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Sulfhydryl Content of Failed and Strophanthin Poisoned Rabbit Hearts. (24289)

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Recently, Bertelli and Mussini(1) reported that the sulfhydryl content of hearts perfused with 1:100,000 strophanthin-g (ouabain) was markedly lowered. There was a decrease of approximately 50% of sulfhydryl content of hearts in complete systolic contracture induced by strophanthin-g, compared to unpoisoned, perfused rabbit hearts. This finding and observations in our laboratory, led us to study the sulfhydryl content of crude myosin extracts of hearts after the hearts had been subjected to various conditions.

Methods. Perfusion. Rabbits of the same strain, 3 to 4 months old, raised in air conditioned animal room on the same diet, were killed by blow on occiput, and their hearts removed as rapidly as possible. These hearts were perfused with the apparatus described by Anderson and Craver(2), available from Metro Industries, L. I. City, N. Y. The perfusion solution was described by Chenoweth and Koelle(3). This work was performed during the summer months. Immediately after removal, the hearts were perfused 30 minutes at 37 $\pm$ 0.5°C. The first 500 ml of perfusion solution passing through heart was not recirculated because it contained blood. To avoid possible injury to hearts by attaching a hook, kymograph recordings were not made. Hearts that appeared damaged or showed evidence of failure during this 30-minute period were not analyzed. After perfusion, the drug was added to a liter of solution in the aeration reservoir, and the heart perfused 15 minutes more. Each heart was perfused 45 minutes. A control group of hearts was perfused 45 minutes continuously. A second group was perfused with 1:100,000 strophanthin Kombé N. F. (S. B. Penick Co.) for 15 minutes. Then they were in complete systolic contracture. A third group was perfused with 0.04% sodium pentobarbital. A fourth group was perfused with 5% ethanol (v/v). A fifth group was failed by perfusing with a solution containing 25% of usual calcium ion concentration: 0.54 mM $CaCl_2$/l, or 0.079 g $CaCl_2 \cdot 2H_2O$/l. All hearts in the last 3 groups failed, stopping in diastole in 7 to 9 minutes; however, all hearts were perfused for the full 15 minute period. *Extraction.* The hearts were removed from apparatus and ventricles were immediately frozen in liquid nitrogen. While still frozen, the ventricles were crushed in stainless steel