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Immunoelectrophoretic Analysis of Serum Proteins in the Edematous Brain Tissue

by

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Many investigations utilizing acidic dyes have been performed in an attempt to unravel the problems of the blood-brain barrier. A primary impermeability of these dyes seems attributable to their protein binding characters. It has been also reported that labeled serum proteins, such as radio-active iodinated serum albumin and fluorescein-labeled serum proteins, are found in the edematous brain tissue. Therefore, it is concluded that serum proteins can penetrate into the brain tissue if the blood-brain barrier is disrupted, whereas they are unable to pass through the unaffected one.

Using electrophoretic method, KAPS (1954), KIYOTA (1959), CUMINGS (1961) and HAUSER et al (1963) reported that relatively increased concentration of albumin was noted in the edematous brain. As HAUSER et al have pointed out, however, there is no definite proof that the protein which was separated electrophoretically by the previously named authors under the name of "albumin" was surely the serum albumin itself, though it was a protein of the same electrophoretic mobility as albumin. It is difficult to examine serum proteins penetrated into the edematous brain tissue independently from intrinsic cerebral proteins, because some of proteins originally existing in the brain tissue are inevitably included in the electrophoregram of the concentrated extracts of the edematous brain tissue.

The purpose of this investigation is, using an immunoelectrophoretic method with the antiserum against serum proteins, to examine whether the serum protein components do truly exist in the edematous brain tissue and, if so, to what extent they take part in the increased proteins.

MATERIALS AND METHODS

Brain edema was experimentally produced in dogs by an extradural compression method described by ISHI et al (1959). Extradural compression was performed for 24 hours and then the experimental dogs were kept alive for another 24 hours without compression. Each dog was sacrificed after 2,000 ml of cold 5% glucose solution had completely been perfused intravascularly from the common carotid arteries on both sides in order to wash out blood in the cerebral vascular bed. In some animals 20 ml of 0.5% trypan blue was given intravenously immediately after the decompression to confirm satisfactory production of edema.
Immediately after removal, brains were kept frozen. In some animals brains were examined without freezing. Blocks of brain tissue measuring $4 \times 4 \times 1$ mm in size were cut out from both grey and white matters on the edematous side as well as from the corresponding parts on the other side for direct immunoelectrophoretic analysis.

Pieces of brain tissues weighing approximately 1 gm each were taken out from the edematous area as well as from the contralateral cerebral hemisphere, homogenized with approximately 2 ml of normal saline, then centrifuged at 10,000 g. Supernatant was concentrated by dialysis against 20 % polyvinyl-pyrolidone solution (approximately 4-fold concentration). These entire manipulations were performed at cold temperature. The concentrated extracts were then analysed immunoelectrophoretically.

Immunoelectrophoresis was carried out with the modified micromethod originally described by Scheidegger (1955). In the agar-plate of 1 mm thickness consisting of 1.25 % agar in veronal buffer pH 8.2, $\mu=0.05$, two kinds of holes were made; one is of $4 \times 4$ mm square in size for tissue blocks, the other, of round being 3 mm in diameter for concentrated extracts. Further technical details have already been reported in the preceding paper. The rabbit antiserum against dog serum proteins was produced by immunizing rabbits with dog whole serum and Freund's complete adjuvant.

RESULTS

The immunoelectrophoretic patterns of serum proteins in the brain tissue are shown in Figs. 1 and 2. Figs. 1 and 2 a are the results of a direct immunoelectrophoretic analysis of the tissue and Fig. 2 b shows the immunoelectrophoresis of extracts obtained.

Fig. 1. a: 8-fold diluted serum. b: edematous grey matter. c: edematous white matter. d: 4-fold diluted serum.
By a direct immunoelectrophoretic analysis of the normal brain tissue no precipitation line can be detected. In cases of the edematous brain, on the other hand, several precipitation lines are recognized; numbers of precipitation lines vary depending upon sorts of animals and parts to be examined, i.e. depending upon the amount of serum proteins to be contained. Larger amount of serum proteins are present in the white matter than in the grey (Fig. 1). Dog serum is diluted with veronal buffer by degrees and examined immunoelectrophoretically. Their patterns are compared with those of direct analysis of tissue. The edematous brain is found to contain serum proteins 1/6 - 1/8 or less in concentration compared with that of serum and its immunoelectrophoretic patterns are more or less similar to those of the diluted serum of corresponding protein contents, though both of them are not exactly identical. Details of this will be described later.

Immunoelectrophoresis of concentrated brain extracts does not always demonstrate distinctly separated precipitation lines, but the patterns of concentrated extracts from the edematous brain tissue are almost identical to those of direct analysis of the tissue. Immunoelectrophoresis of concentrated extracts of normal brain tissue demonstrates no precipitation line at all or in some cases only slight turbidity in albumin and beta-globulin region.

The most conspicuous difference on the immunoelectrophoretic pattern of the edematous brain tissue from that of diluted serum is the presence of unusual precipitation lines in the alpha-2- to beta-1-region around the starting well. These precipitation lines are continuous with those in the other regions. The most distinct example is albumin. In Fig. 1 and 2 a, it is observed that the precipitation line of albumin is continuous with one of these precipitation lines in the alpha-2- to beta-1-region. In the immunoelectrophoretic pattern of the edematous brain extract (Fig. 2 b), one of the precipitation lines in the alpha-2- to beta-1 region is recognized to show the reaction of partial identification with that of albumin. The precipitation line of alpha-1 lipoprotein shows the same characteristics (Fig. 3). The precipitation line around the starting well which is connected with that of alpha-1 lipoprotein takes more anodic position than that part of albumin. The most cathodic component of gamma-globulin appears to decrease in amount or even to be absent in the edematous brain tissue. As shown in Fig. 2, the precipitation lines of beta-2- and gamma-globulins in the alpha-2- to beta-1- region are situated in close proximity to the antiseraum trough.
DISCUSSION

From our experiment it is demonstrated that in normal brain tissue serum proteins cannot be detected immunoelectrophoretically after intravascular perfusion of cold glucose solution, but a considerable amount of serum proteins are proved to exist in the edematous portions of the brain. There is possibility, therefore, that all of the serum proteins may enter unselectively into the brain tissue when it is edematous and, in addition to this, some evidences which may indicate breakdown of these serum proteins in the edematous brain tissue are also observed.

Recently labeled proteins, such as radio-iodinated serum albumin and fluorescein-labeled proteins, have been widely used for the purpose of investigating impermeability of the serum proteins through the cerebral vessel walls. Previously, however, various kinds of acidic dyes, commonly trypan blue, were used for the study of bloodbrain barrier. Because of their rapid binding to serum albumin, it has been generally believed that they are the indicators of albumin entry into the brain tissues of various pathological conditions, but, precisely speaking, it is somehow uncertain for these dyes to indicate albumin entry in pathological conditions. CUTLER et al (1964) discussed on this problem in detail.

With help of these indicators, many authors have reported that serum proteins can penetrate into the brain tissue in pathological states. KLATZO et al (1962) reported that no conclusive difference could be found in the morphological appearance between fluorescein-labeled albumin and fluorescein-labeled gamma-globulin in the edematous brain induced by cold injury. It may suggest that in the edematous brain tissue all of the serum protein constituents are present in the similar fashion. These investigations using labeled proteins, however, have some disadvantages; first of all labeled proteins are not identical to natural proteins; secondly in most cases of animal experiments they are heterologous proteins; and in addition they cannot be detected by proteins themselves but only by their labels.

Paper-electrophoresis revealed an increase of albumin in the extract of the edematous brain. It was considered to be caused by migration of serum albumin into the edematous brain tissue, but there was no definite proof that increased albumin was of serum origin. Besides, it was not clear from the paperelectrophoretic analysis whether serum proteins other than albumin were also present or not. By the use of the specific antiserum against
serum proteins immunoelectrophoresis is the definite method to find out whether proteins are of serum origin or not and to detect only serum proteins in the extractable proteins of brain tissues.

The immunoelectrophoretic patterns of the edematous brain tissue of high degree are similar to those of 6 to 8 fold diluted serum. This fact is to be interpreted that the amount of serum proteins contained in the edematous brain tissue is quantitatively equivalent to that of 6 to 8 fold diluted serum. In the region of alpha-2 to beta-1 globulin there are precipitation lines which are not recognized in immunoelectrophoretic patterns of serum, the most distinct one of which is connected with that of albumin. Namely it means that this particular precipitation line located in the region of alpha-2 to beta-1 is given by the protein that is antigenitically identical with albumin. The sudanophilic precipitation line which takes slightly more anodic position is also antigenitically the same as alpha-1-lipoprotein.

For the correct interpretation of these results it must be taken into consideration whether the direct immunoelectretic analysis of tissue is methodologically significant or not. There may be some possibility that tissue in the starting well interferes electrophoretic separation of proteins within it. However, immunoelectrophoresis of homogenized brain tissue and concentrated brain extracts shows almost the same immunoelectrophoretic patterns. It may well be considered, therefore, that the presence of a tissue in a starting well does not interfer electrophoretic migration of proteins.

Generally gamma-globulin gives the characteristic precipitation line extending from gamma- to alpha-2 region. Near the starting well, i.e. in the alpha-2 to beta-1 region it consists of higher molecular component than that of cathodic part and its precipitation line in this region lies at a distance from the antiserum trough. Immunelectrophoretic study of serum proteins in cases of the edematous brain tissue, however, reveals that gamma-globulin gives the precipitation line close to the antiserum trough (Fig. 2). The same tendency is also recognized on beta-1- and beta-2-globulins. The above mentioned fact means that the part of gamma-globulin which has the same electrophoretic mobility as alpha-2- or beta-1-globulins is more easily diffusible or in another word, of less molecular weight. It is thought that gamma-globulin and perhaps other serum proteins too, are enzymatically broken down upon entering into the brain tissue, and that considerable parts of splitted proteins still have their native antigenic characters.

KAPS, KIYOTA and CUMINGS reported that paperelectrophoretically there was an increase in albumin in extractable proteins of the edematous brain tissue. KIYOTA (1959) considered that an increase in albumin was due to migration of serum albumin into edematous brain and that the increased alpha-globulin fraction was possibly due to denaturation of tissue proteins. According to HAUSER et al (1963) an increase in albumin corresponds to approximately 50 per cent of the total increase in extractable proteins. The present study reveals that in addition to albumin a considerable amount of serum proteins in the region of alpha-2 to beta-1 is found in the edematous brain tissue. It may be safely concluded that the increased albumin in the edematous brain tissue is derived from the serum and that some splitted serum proteins may contribute considerably to an increase of alpha- and/or beta-globulin in extractable proteins of the edematous brain.
Among the serum proteins minor constituents, such as macroglobulins, are failed to be detected immunoelectrophoretically in the edematous brain tissue. The high-molecular serum proteins such as beta-2-macroglobulin and beta-1-lipoprotein were only found in the cerebrospinal fluid of various central nervous system diseases. They were considered to enter into the brain tissue at the site of pathological processes because of the disrupted blood-brain barrier and then to appear in the cerebrospinal fluid, as reported previously. In this regard it is interesting to find these minor serum protein constituents in the brain tissue. For this purpose more sensitive method is naturally required, and we are now working on this problem.

REFERENCES

和文抄録

脳組織内の血清蛋白について——免疫電気泳動的研究

京都大学医学部脳神経外科（主任：半田・佐教授）

染 田 邦 幸，景 山 直 樹

犬に於て硬膜外压迫法により脳浮腫を作製し、脳組織内の血清蛋白を免疫電気泳動法により検査した。組織片を直接寒天板の孔に入れて電気泳動を行ない、比を更に全血清に対する抗血清を用いて応ぜしめた場合、正常脳では全く沈降線は出現しないが、浮腫脳では血清を1/6～1/8に稀釀した場合とほぼ同じ沈降線像を呈する。すなわち、浮腫脳中には、最も多い所で、血清の1/6～1/8程度の濃度で血清蛋白が存在する

ことを示しており、又血清中の各蛋白成分が、無差別に組織内に入り込むと云える。アルブミンやβ-リボ蛋白が、α1～3領域に沈降線を作つてくるが、これは組織内で抗元性は変化しないが、電気泳動度が変わる程度に分離されたためではないかと考えられる。脳組織より可溶性蛋白を抽出濃縮して行った実験でもほぼ同様の結果が得られた。