

um or ATP were without effect in reversing the effects of diethylstilbesterol, dienes-
terol or hexosterol on mitochondrial swelling.
The relatively large quantities of inhibitors
needed to produce significant changes in
mitochondrial volume together with a lack of
correlation of the relative abilities of these es-
trogenic substances to produce swelling and
potentiate 6-aminonicotinamide toxicity on
the 755 tumor¹ apparently discount possible
in vivo changes in mitochondrial volume pro-
duced by these compounds, as being pertinent
to the biological results. Thus, subsequent
increases in mitochondrial permeability to 6-
aminonicotinamide or pyridine nucleotide
analogues containing 6-aminonicotinamide
may be ruled out as a major factor in en-
hancing 6-aminonicotinamide antagonism of
intramitochondrial enzymes dependent on py-
ridine nucleotide.

¹ Diethylstilbesterol and hexosterol are far more
effective than estradiol, dienes-terol and phloretin in
potentiating the anti-tumor activity of the niacin
antagonist 6-aminonicotinamide in the 755 tumor
grown in 755 black mice (unpublished data, Dietrich,
L. S. and Martin, D. S.).

Summary. The ability of the compounds
diethylstilbesterol, hexosterol, dienes-terol,
phloretin and estradiol to induce swelling of
liver mitochondria was determined. Diethyl-
stilbesterol, hexosterol and dienes-terol pro-
duced marked mitochondrial swelling at final
concentration of $4-8 \times 10^{-5}$ M. The com-
pounds estradiol and phloretin were inactive
at the highest levels tested (8×10^{-5} M).
Under the experimental conditions ATP,
Mg⁺⁺ or versene did not prevent the mito-
chondrial swelling induced by diethylstilbes-
terol.

1. Martin, D. S., Dietrich, L. S., Fugman, R. A.,
PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 58.
2. Dietrich, L. S., Martin, D. S., *Cancer Res.*, 1961,
v21, 361.
3. Tapley, D. F., *J. Biol. Chem.*, 1956, v222, 325.
4. Packer, L., *ibid.*, 1960, v235, 242.
5. Raaflaub, J., *J. Helv. Physiol. Acta*, 1953, v11,
142.
6. Packer, L., Bacila, M., *Acta Physiol. Latino
Am.*, 1958, v8, 230.
7. Blecher, M., White, A., *J. Biol. Chem.*, 1960,
v235, 3404.

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Proteins in Normal Cerebrospinal Fluid Not Found in Serum.* (26569)

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Recent reports dealing with proteins in the
cerebrospinal fluid (CSF) have neither proved
nor disproved the existence of proteins specific
for CSF and not found in serum. Paper elec-
trophoresis(1,2,3) does not demonstrate anti-
genic differences between the 2 fluids. The
commercially available antiserum against hu-
man serum proteins has been used in several
immuno-electrophoretic investigations of CSF
(see survey by Burtin(4) and by Clausen
(5)). These investigations, however, did not
reveal the existence of special CSF proteins
not found in serum. Immuno-electrophoresis
using specific sera against CSF is a reliable

method of demonstrating differences between
proteins of CSF and proteins of serum. This
communication adds some data to the very
few results obtained in this field(6).

Materials and methods. Normal cerebro-
spinal fluids were obtained from patients who
had undergone pneumo-encephalography at
The Municipal Hospital, Copenhagen, Den-
mark. These patients had no other neurologi-
cal complaints than cephalalgia, without ob-
jective findings.

Immuno-electrophoresis was performed as
described by Scheidegger(7). The antisera
made in this laboratory were manufactured as
described previously(8). The following anti-
sera were used: 1) Rabbit anti CSF anti-

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ple Sclerosis, Copenhagen, Denmark.

serum (No. 11), made in this laboratory. 2) Rabbit anti human gamma globulin antiserum from Behringwerke Marburg/Lahn, Germany.

The CSF used for immunization and immuno-electrophoresis was pooled from several persons and concentrated to 5 g% (w/v) by suction dialysis in an apparatus and membranes from Membrangesellschaft, Göttingen, Germany.

The 5 CSF gamma globulin fractions, found in agar gel micro-electrophoresis especially distinctly in CSF from patients with infectious diseases, were demonstrated with agar gel micro-electrophoresis of CSF from a patient with lymphocytic meningitis as described by Loewenthal *et al.* (9).

Results. Fig. 1 shows the immuno-electrophoresis of normal CSF developed with specific antiserum No. 11. The findings are correlated to similar tracings obtained when No. 11 developed normal human serum.

One prealbumin line, one albumin line, and several precipitation lines in the alpha- and beta-areas corresponding to similar lines in human serum immuno-electrophoresis can be seen. These lines are neither visualized in immuno-electrophoresis when antiserum 11 is absorbed with normal human serum nor when CSF is developed with specific anti gamma globulin. Thus all the precipitation bows in the prealbumin, albumin, and alpha-areas correspond to antigenically closely related proteins in human serum. On the other hand, 2 precipitation lines in the beta- and gamma-areas, called beta-CSF and gamma-CSF, can also be developed in immuno-electrophoresis with anti-CSF 11 absorbed with normal human serum. This means that these 2 proteins might be specific for CSF.

The precipitation line corresponding to beta-CSF corresponds to a group of antigenically identical proteins with different mobilities, from the slow albumin zone to the intermediate gamma globulin area.

Gamma-CSF has a small interval of mobility from the intermediate gamma-area to a mobility somewhat slower than the slowest gamma-mobility in serum.

Discussion. Previous investigations seem to indicate differences between CSF and human serum, for example, the tau-fraction

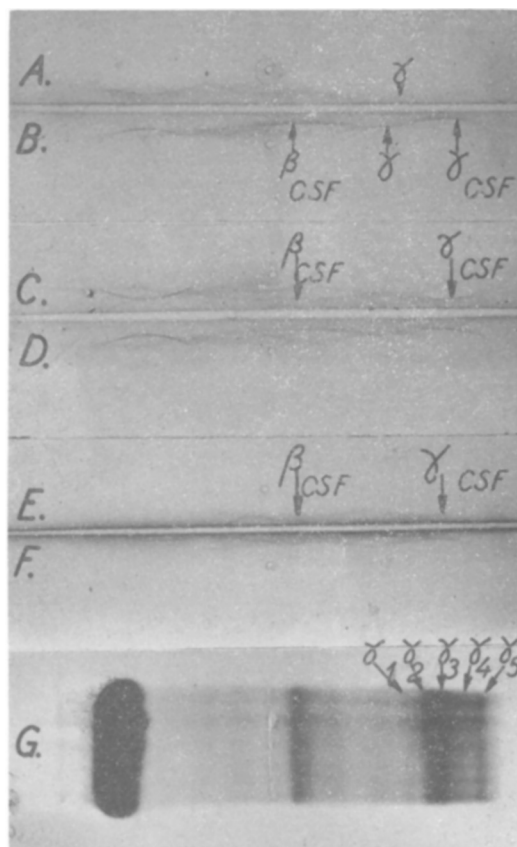


FIG. 1. Immuno-electrophoretic findings in normal cerebrospinal fluid. Demonstration of 2 specific proteins in CSF by means of a specific anti-CSF antiserum (No. 11). A. Immuno-electrophoresis of normal human serum developed with the anti-CSF antiserum No. 11. Only one precipitation line corresponding to the conventional serum gamma globulin in gamma area is seen. B. Normal concentrated CSF developed as A. Beside the serum gamma precipitation line precipitation lines corresponding to beta-CSF and gamma-CSF are visualized. C and D. Same tracings as B. E and F. Same tracings as C and D developed with anti-CSF antiserum absorbed with normal human serum in the proportion 3:1. Precipitation lines corresponding to the 2 specific proteins: beta-CSF and gamma-CSF are seen selectively. G. Agar gel micro-electrophoresis of CSF from a patient with lymphocytic meningitis, demonstrating the 5 gamma globulin fractions, of which some might correspond to the gamma-CSF fraction found in immuno-electrophoresis.

found in paper electrophoresis(10), and the 5 gamma globulin fractions in CSF revealed in agar gel micro-electrophoresis(11). These investigations could not, however, show any possible antigenic differences between the proteins of the 2 media. Immuno-electrophoresis has shown that the tau-fraction possibly cor-

responds to enzymatically changed transferin, which has preserved the antigenic properties involved in the immuno-electrophoretic procedure(12,13,14).

Chevance, Galli, Jeanmaire, and Gerard(6) have recently described an immuno-electrophoresis with specific CSF antiserum. They have described 9 precipitation lines, among them a slowly moving gamma globulin. However, as mentioned by Burtin, the explanation of the immuno-electrophoretic findings was limited by lack of a potent antiserum. The data given here support the findings by Chevance *et al.*(6) of a slowly moving fraction in CSF with a mobility slower than that of the serum gamma globulin, antigenically different from serum gamma globulin. Both the absorption experiments with normal human serum and the crossing of the 2 slowest moving precipitation lines seem to exclude a very close antigenic relation between serum gamma globulin and gamma-CSF (Fig. 1). These findings may account for some of the slowest moving gamma globulin fractions revealed in agar gel micro-electrophoresis and explain the selective increase of these in diseases of the central nervous system (Fig. 1).

Summary. Immuno-electrophoresis of normal concentrated cerebrospinal fluid, developed with a specific rabbit anti cerebrospinal fluid antiserum, has demonstrated the exist-

ence in the gamma and beta areas of 2 proteins not found in serum.

1. Kabat, E. A., Landow, H., Moore, D. H., *Proc. Soc. Exp. Biol. and Med.*, 1942, v49, 260.
2. Kabat, E. A., Moore, D. H., Landow, H., *J. Clin. Invest.*, 1942, v27, 571.
3. Grabar, P., Williams, C. A., *Biochim. biophys. Acta*, 1953, v10, 193.
4. Burtin, P., in *Analyse Immuno-électrophorétique*, Grabar, P., Burtin, P., Ed., Masson et Cie, 1960, 245.
5. Clausen, J., *World Neurol.*, 1960, v6, 479.
6. Chevance, L. G., Galli, A., Jeanmaire, J., Gerard, G., in *Analyse Immuno-électrophorétique*, Grabar, P., Burtin, P., Ed., Masson et Cie, 1960, 253.
7. Scheidegger, J. J., *Internat. Arch. Allergy*, 1955, v7, 103.
8. Heremans, J., Clausen, J., Heremans, M. Th., Rask-Nielsen, R., *J. Nat. Cancer Inst.*, 1959, v22, 45.
9. Loewenthal, A., Karcher, D., Van Sande, M., in *Protides of the Biological Fluids*, Peters, H., Ed., Elsevier Publ. Comp., 1960, 309.
10. Bücher, Th., Matzelt, D., Pette, D., *Klin. Wschr.*, 1952, v30, 325.
11. Loewenthal, A., Van Sande, M., Karcher, D., *J. Neurochem.*, 1960, v6, 51.
12. Pette, D., Stupp, I., *Klin. Wschr.*, 1960, v38, 109.
13. Clausen, J., *Acta Psych. Scand.*, 1960, v35 (Suppl. 148), 11.
14. Clausen, J., Munkner, T., *Nature*, 1961, v189, 60.

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A Study of the Mechanism of the Increase in Cardiac Output Induced by Isopropylarterenol Hydrochloride (Isuprel®).^{*} (26570)

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Many investigators(1-5) have studied the effect of isopropylarterenol hydrochloride (Isuprel®) on various aspects of the circulation and have demonstrated that the drug increases heart rate and cardiac output, decreases mean arterial blood pressure or leaves it unchanged, and lowers total peripheral resistance. To evaluate the role of the rise in heart rate normally induced by Isuprel in the

genesis of the increase in cardiac output caused by the drug, the effect of Isuprel on

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