MATERIAL AND METHODS

The case material consisted of all consecutive cases of acute cerebrovascular accidents (based on Chusid's criteria, 1972), meningitides and encephalitides (diagnosed on the basis of clinical examination and routine cerebrospinal fluid examination) and normal controls (cases with no neurological, cardiac, skeletal, muscular and renal diseases likely to affect the transaminase and lactic dehydrogenase levels) presenting in the emergency and/or medical wards of M.L.E. Medical College and Hospital, Jhansi. Informed consent to the investigation was taken in all cases.

Acute myocardial infarction, hepatic, renal and skeletal muscle diseases, head injury and the diseases which result in a documented rise in the level of GOT, and LDH were excluded from this study.

All the neurological cases under study were subjected to a thorough interrogation and clinical examination. All the cases as well as normal controls were investigated as below:

1. Urine examination for sugar, albumin and microscopic findings.
3. Blood sugar (fasting and postprandial) measurement.
6. Serum cholesterol measurement.

METHODOLOGY:

Immediately after hospitalisation cerebrospinal fluid samples were obtained. Following lumbar puncture, a blood sample was also collected, soon after, in each case. Cerebrospinal fluid was centrifuged and separated from any sediment. Serum was separated from the clot within an hour after the collection of blood sample. Serum and supernatant C.S.F. were stored at 0°C until enzyme estimation was done, which was not more than 48 hours in any case. Traumatic spinal fluid specimens were discarded.

The C.S.F. and serum GOT levels were estimated by the colorimetric method of Reitman and Frankel (1957), as outlined below.

The C.S.F. and serum LDH levels were estimated by the colorimetric method as described by Westman.

The serum and C.S.F. enzyme estimations were repeated as and when necessary to determine the variations in enzyme activity during period of follow up.
ESTIMATION OF G.O.T.

Principle:
Transfer of an aminogroup from an amino acid to an alpha keto acid is an important step in the metabolism of amino acids. Two enzymes occur in human tissues which catalyse reactions of this type. These are glutamic oxaloacetic transaminase (GOT, Aspartate amino transferase) and glutamic pyruvic transaminase (GPT, Alanine amino transferase). G.O.T. is involved in the following transamination:

\[
\text{alpha ketoglutaric acid} + \text{Aspartic acid} \xrightarrow{\text{GOT}} \text{glutamic acid} + \text{oxaloacetic acid}.
\]

The oxaloacetate formed in the reaction with G.O.T. decarboxylates spontaneously to pyruvate. This pyruvate reacts with 2,4-dinitrophenylhydrazine which is measured in the colorimeter at 510 mili microns.

Method:
(Atimian and Frankel, 1957).

Reagents:
G.O.T. substrate - (200 mM-DL-aspartic acid; 2mM-alpha-ketoglutarate).

13.3 g of DL aspartic acid was dissolved in the minimum amount of 0.15M sodium hydroxide which dissolved it and a solution was produced with a pH of 7.4. About 95 ml was required. 0.346 g of alpha ketoglutaric acid was
added and dissolved by adding a little more sodium hydroxide. The pH was adjusted to 7.4 and the solution was made to 500 ml with phosphate buffer. It was divided into 10 ml portions and stored frozen at -15°.

**Stock pyruvate standard:**

(20 ml) 230 mg of sodium pyruvate was dissolved per 100 ml of phosphate buffer. It was stored at -15° in 1 ml aliquots.

**Working pyruvate standard:**

(4 ml), the stock standard was diluted 1 in 5 with phosphate buffer and stored at -15°. It was prepared fresh each week.

**2,4-dinitrophenyl hydrazine:**

(1 ml) 19.6 mg of dinitrophenyl hydrazine was dissolved in 10 ml of concentrated hydrochloric acid and made to 100 ml with water. It was kept at room temperature in a brown bottle.

**0.4 M-sodium hydroxide:**

16 g of sodium hydroxide per litre in water.

**Phosphate buffer:**

(pH 7.4), 11.3 g of dry anhydrous disodium hydrogen phosphate and 3.7 g of dry anhydrous potassium dihydrogen phosphate per litre in water. The pH was checked and it was stored at 0°.
Test:

0.5 ml of substrate was warmed in a water bath at 37° for 3 min. 0.1 ml of serum/CSP was added, mixed and incubated for exactly 60 min. The tubes were removed from the bath, 0.5 ml of DNPH solution was added immediately and was mixed well.

Control:

0.5 ml of substrate was mixed with 0.5 ml of DNPH solution and 0.1 ml of serum/CSP was added.

Standard:

0.1 ml of working pyruvate standard was mixed with 0.4 ml of substrate, 0.1 ml of water and 0.5 ml of DNPH solution.

Blank:

0.5 ml of substrate, 0.1 ml of water and 0.5 ml of DNPH were mixed in a test tube.

The DNPH was allowed to react in all tubes for 20 min. at room temperature, then 5 ml of 0.4 N-sodium hydroxide was added, mixed well and left for a further 10 min.

The pyruvate formed by the serum/CSP was responsible for the difference between test and control (T-C). The pyruvate in 0.1 ml of working standard (0.4 micromole) produced the difference between standard and blank (S-C), so the pyruvate formed in 42 min. by 0.5 ml of serum/CSP was 1
\[
\frac{T-C}{S-S} \times 0.4 \text{ micro mole}
\]

Thus the pyruvate formed per min. per litre of serum/CSF was:

\[
\frac{T-C}{S-S} \times 0.4 \times \frac{1}{60} \times \frac{1000}{0.1}
\]

\[
= \frac{T-C}{S-S} \times 67 \text{ micro mole}
\]

The calculated pyruvate was converted into I.U. per litre by Wootton's reference table.

**ESTIMATION OF LADH-:**

**Principle:**

Lactic dehydrogenase is an enzyme of almost universal distribution in the body which catalyses the reversible transformation of pyruvate to lactate.

\[
\text{Pyruvic Acid} + \text{NAD}^+ \rightarrow \text{Lactate} + \text{NADH}
\]

Pyruvate is reduced by incubation with serum or CSF in the presence of enzyme NADH. The reaction is stopped by adding DME solution which reacts with the remaining pyruvate forming a hydrazine. The amount of unreacted pyruvate is found by measuring the brown colour, produced when the hydrazine is made alkaline. The determination is performed at 540 because some of the serum/CSF is very sensitive to heat.

**Initial:**

**Reagents:**
Buffer:
(pH 7.4) 11 g of anhydrous disodium hydrogen phosphate and 2.7 g of anhydrous potassium dihydrogen phosphate per litre in water.

Stock sodium pyruvate:
(37.3 mM) 415 mg of sodium pyruvate buffer. It was divided into 1 ml samples and kept at -15°C.

Working sodium pyruvate buffered substrate:
(0.75 mM). Stock pyruvate solution was dissolved 1 in 50 with phosphate buffer. Fresh dilutions were made daily.

Reduced nicotinamide adenine dinucleotide (NADH):
10 mg NADH per ml of phosphate buffer. It was made fresh for each batch of tests.

2,4-dinitrophenylhydrazine:
(2 mM) 400 mg of dinitrophenylhydrazine was dissolved in 35 ml of concentrated hydrochloric acid. It was made up to 1 litre with water and stored in a dark bottle.

0.4 N sodium hydroxide:
16 g of sodium hydroxide per litre of water.

Method:
Test:
1 ml of buffered substrate was mixed with 0.1 ml of enzyme or CNP. The mixture was placed in a water bath at 37°C. After a few minutes the reaction was stopped.
adding 0.1 ml of \( \text{NADH}_2 \) solution. It was incubated for exactly 15 min. the test tube was removed from the bath and 1 ml of \( \text{DNPH} \) solution was added immediately with mixing.

**Control:**

1 ml of substrate, 0.2 ml of buffer and 1 ml of \( \text{DNPH} \) solution.

**Blank:**

1.2 ml of buffer and 1 ml of \( \text{DNPH} \) solution. All the tubes were allowed to stand at room temperature for 20 min.

10 ml of 0.4 N sodium hydroxide solution was added to each and mixed. The coloured solutions were compared at 510 milli micron after 10 min.

The control tube contained 0.75 micro mole of pyruvate.

Amount of reacted pyruvate was \( \frac{C_{\text{Cr}}}{C_{\text{Cr}}^0} \times 0.75 \) micro mole.

This was the effect of the enzyme in 0.1 ml of serum or CSF acting for 15 min. The pyruvate reacting per minute per litre of serum/CSF was thus:

\[ \frac{C_{\text{Cr}}}{C_{\text{Cr}}^0} \times 0.75 \times \frac{1}{15} \times \frac{1000}{0.1} \]

\[ \text{LDH} = \frac{C_{\text{Cr}}}{C_{\text{Cr}}^0} \times 500 \text{ (micro mole per min., per litre)} \]