had similar headaches independently. Similarly, a recent study demonstrated a connection between prior migraine and headache during transient global amnesia. It is possible that ischemia in the region of susceptible blood vessels may trigger components of the migraine cascade in vulnerable individuals. Alternatively, Edmeads postulated an important role for platelet adhesion, aggregation and release in headache of atherothrombotic disease, processes which have been implicated in the production of migraine headache. Though the underlying pathophysiology of pain in both these conditions remains to be elucidated, a common process in some patients is implied by our data.

References

Moderate Homocysteinemia — A Possible Risk Factor for Arteriosclerotic Cerebrovascular Disease

LARS E. BRATTSTROM, M.D., JAN ERIK HARDEBO, M.D., AND BJORN L. HULTBERG, M.D.

SUMMARY Highly elevated concentrations of homocysteine measured as homocysteine or cysteine-homocysteine mixed disulfide (MDS) are found in plasma and urine in subjects with inherited abnormalities of the methionine metabolism. These subjects have a high incidence of arteriosclerotic vascular complications during childhood. Homocysteine causes endothelial cell injury and cell detachment that initiates the development of arteriosclerosis. The present study demonstrates a significantly elevated mean plasma MDS concentration in 19 patients with arteriosclerotic cerebrovascular disease compared to 17 controls. Our findings suggest that moderate homocysteinemia might be a risk factor for arteriosclerotic cerebrovascular disease.

HOMOCYST(E)INEMIA/URIA IS usually secondary to deficiency of cystathionine β-synthase, a pyridoxal phosphate dependent enzyme in the transsulfuration pathway (fig. 1). It is an autosomal recessive inborn error of methionine metabolism. Homocysteinemia can also be due to a reduced capacity for homocysteine remethylation via the folate and cobalamine dependent transmethylation pathway. It is characterized by elevated concentrations of homocysteine and its two disulfides homocysteine and cysteine-homocysteine mixed disulfide (MDS) in plasma and urine.

The main cause of morbidity and mortality in homocysteinemic patients is progressive premature arteriosclerosis and associated thromboembolic complications. Histopathologically there are widespread arterial focal lesions with fibrous intimal plaques and medial fibrosis with fraying and splitting of the internal elastic membranes. Homocysteine or its derivates are considered to cause these changes.

Experimental studies with homocysteine thiolactone in rabbits have in some but not all studies induced arteriosclerotic lesions. Continuous infusion of bafoons with homocysteine thiolactone caused patchy...
endothelial desquamation of the aorta and typical intimal arteriosclerotic lesions in all infused animals. Experimental homocysteinemia in pigs, however, produced no detectable lesions. In vitro homocysteine caused cell injury and cell detachment in cultured human umbilical venous endothelial monolayers. Endothelial cell injury has been postulated to be the initial event in the pathogenesis of arteriosclerosis.

Wilcken et al 1976 found detectable concentrations of plasma MDS, indicative of homocysteine, in 17 of 25 men with coronary artery disease and in 5 of 22 controls 4 hours after a 100 mg/kg body weight methionine load. The study raised the possibility that mild homocysteinemia might play a role in the pathogenesis of arteriosclerosis. However, this has been contested in a later study by Wilcken et al 1983 in which no difference was found in plasma homocysteine levels between patients with ischemic heart disease and controls.

In the present study plasma MDS, methionine and cystine were measured after an overnight fast and 4 hours after a methionine load in patients with arteriosclerotic cerebrovascular disease and in control subjects.

Patients and Methods

We studied 19 patients, 10 men and 9 women aged from 34 to 59 years and 17 healthy volunteers, 10 men and 7 women aged from 34 to 63 years. The patients were at medical care in hospital due to TIA or minor strokes and were not studied in the acute phase of their disease. One female patient however, with a progressive stroke was studied within one week from onset of symptoms. Sixteen of 19 patients had angiographic or Doppler scanning evidence of internal carotid artery stenosis or occlusion. All control subjects were free of previous or actual symptoms indicating vascular disease. Physical examination revealed no bruits over main arteries and peripheral pulsations were found normal.

Medical histories, smoking habits, weight, height, blood pressure and current drug therapy were recorded. Fasting serum cholesterol, serum triglycerides, serum creatinine, serum folate, serum cobalamin and blood glucose were measured by standard techniques. Fourteen patients were on one or more medications (dicumarol, digoxine, nifedipine, hydralazine, verapamil, β-blockers, thiazids, spironolactone or ASA). No medications were administered 12 hours prior to the investigations.

On the day of study a heparinized venous blood sample was drawn at about 8 a.m. after an overnight fast. L-Methionine 100 mg/kg body weight was then given orally in about 200 ml of orange juice. The fast continued and 4 hours later a second blood sample was drawn. The samples were immediately centrifuged at 4°C and plasma was stored at −70°C until analysis. From the female patient with a progressive stroke only the fasting sample was drawn and no methionine was given.

Before analysis the samples were quickly thawed in cold water and then immediately deproteinized with sulphasalicylic acid 150 mg/1.5 ml plasma plus 1.5 ml lithium buffer (pH 2.2). The samples were then centrifuged and the supernatants were again centrifuged and the final supernatants were analysed using a JEOl Amino Acid Analyser (Modell JLC-6AH) and a buffer system described by Jeppsson and Karlsson 1972. Norleucine was used as an internal standard. Concentrations for MDS, methionine and cystine were calculated in the two groups. Statistical significance was assessed by Student’s t-test and Mann-Whitney rank sum test for unpaired data. For correlation between different parameters linear regression analysis was used and the r-values were tested with the t-test. A p-value less than 0.05 was considered significant.

Results

The study population is described in table 1. Patients were in average 1.8 years older than controls. Mean values for height, weight and body surface area were lower in patients also after correction for the larger number of women in the patient group. Mean serum cholesterol and serum triglycerides were higher in patients, but only one patient had cholesterol above 7 mmol/l and three patients had triglycerides above 2.1 mmol/l. Mean values for serum folate and serum cobalamin were found higher in patients. Serum folic acid was within normal range in all subjects, but one patient had serum cobalamin below normal (Normal 110–650 pmol/l). Serum creatinine was normal in all subjects. One patient had a fasting blood glucose of 6 mmol/l but no manifest diabetes. There was a large predominance for smokers in the patient group and 10 of 19 patients had a known hypertension but none of the controls.

Mean values for methionine dosage in g/m² body surface area and for plasma concentrations of methionine, MDS and cystine before and after per orally loading with methionine in patients and controls are given in table 2. As methionine was given per kg body
weight, the dosage per m² body surface area became individually different, and were in average 0.1 g/m² lower in patients. No differences were found for the mean pre and post load plasma methionine concentrations between the groups. After the load there was more than a 20 fold increase in plasma methionine in most subjects. No subject had a low post load methionine concentration corresponding to poor absorption.

The mean plasma MDS concentrations were significantly elevated both before (p < 0.05) and 4 hours after (p < 0.05) the methionine load in the patient group compared to the control group. The individual values are plotted in figure 2. In all subjects there was a rise in MDS concentrations after the load. The correlation between the MDS values pre and post load was significant (r = 0.718, p < 0.001) indicating that MDS values after an overnight fast also reflect the capacity to metabolise methionine when this pathway is stressed (fig. 3). Mean plasma cystine concentrations were slightly elevated both before and after the methionine load in patients and there was no significant elevation of the concentrations after the load.

The correlation between the given dosages of methionine g/m² body surface area and the post load plasma methionine concentrations was significant (r = 0.624, p < 0.001) but between the given dosages and the post load plasma MDS concentrations there was no correlations (fig. 4). This discrepancy suggests that the given dosage saturates one of the metabolic steps prior to homocysteine in the transsulfuration pathway. No pattern was found between received medications and the plasma amino acid concentrations. Smokers and patients with hypertension were evenly distributed among subjects with high and low concentrations of MDS. There was no correlation between MDS and serum cholesterol or triglycerides.

### Discussion

The present study demonstrates increased plasma homocysteine measured as MDS both after an overnight fast and after a methionine load in some patients with arteriosclerotic cerebrovascular disease. As homocysteinemia is associated with premature atherosclerosis and as experimental studies both in vivo and in vitro show that homocysteine causes endothelial cell injury and cell detachment these data suggest that homocysteine might have contributed to the cerebrovascular disease in these patients as an additive risk factor.

For ethical reasons current medications were not stopped several days before the study. Consequently the results have to be interpreted with caution. However, elevated MDS concentrations were also found in patients without any medication. The kinds of drugs administered to patients with high MDS values were also given to patients with low MDS values. A previous study found no difference in MDS concentrations between patients with chronic renal failure receiving and not receiving a variety of antihypertensive agents and diuretics. Therefore the influence of medications seems to be of minor importance but cannot be excluded.

Wilcken et al 1983 could not reproduce their results from 1976 in patients with ischemic heart disease. On reviewing their earlier data they discovered that high MDS values were obtained in overweight patients who received larger amounts of methionine per m² body surface area since the loads were given per kg body weight. In our study we found no correlation between given dosages of methionine g/m² body surface area and the post load MDS concentrations. This is in conflict with their arguments.

The two patients with the most elevated plasma MDS concentrations in our study also had the most

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**Table 1** Mean Values ± S.E.M. of Relevant Data for Control Subjects and Patients with Arteriosclerotic Cerebrovascular Disease. Number of Smokers and Subjects with Hypertension in the Two Groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (N = 17)</th>
<th>Patients (N = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.5 ± 2.4</td>
<td>49.3 ± 1.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>71.5 ± 3.4</td>
<td>65.6 ± 3.0</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.3 ± 2.2</td>
<td>168.2 ± 2.5</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.84 ± 0.05</td>
<td>1.74 ± 0.05</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>5.2 ± 0.3</td>
<td>5.5 ± 0.3</td>
</tr>
<tr>
<td>Serum homocysteine (mmol/l)</td>
<td>1.1 ± 0.1</td>
<td>1.5 ± 0.3</td>
</tr>
<tr>
<td>Serum folate (mmol/l)</td>
<td>11.8 ± 1.3</td>
<td>13.3 ± 1.6</td>
</tr>
<tr>
<td>Serum cobalamine (pmol/l)</td>
<td>356.1 ± 33.3</td>
<td>436.4 ± 57.2</td>
</tr>
<tr>
<td>Smokers (N)</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Hypertension (N)</td>
<td>0</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 2** Mean Values ± S.E.M. for Methionine Dosage per m² Body Surface Area, Plasma Concentrations of Methionine, Cysteine-Homocysteine Mixed Disulfide (MDS) and Cystine after an Overnight Fast and after a Methionine Load (100 mg/kg) in Control Subjects and Patients with Arteriosclerotic Cerebrovascular Disease

<table>
<thead>
<tr>
<th></th>
<th>Methionine dosage (g/m²)</th>
<th>Methionine fasting (µmol/l)</th>
<th>Methionine 4h post load (µmol/l)</th>
<th>MDS fasting (µmol/l)</th>
<th>MDS 4h post load (µmol/l)</th>
<th>Cystine fasting (µmol/l)</th>
<th>Cystine 4h post load (µmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (N = 17)</td>
<td>3.9 ± 0.1</td>
<td>25.8 ± 1.8</td>
<td>664.5 ± 37.6</td>
<td>3.5 ± 0.2</td>
<td>11.9 ± 1.0</td>
<td>56.9 ± 2.5</td>
<td>60.7 ± 1.9</td>
</tr>
<tr>
<td>Patients (N = 19 pre load, N = 18 post load)</td>
<td>3.8 ± 0.1</td>
<td>27.3 ± 1.8</td>
<td>663.5 ± 23.5</td>
<td>5.1 ± 0.6*</td>
<td>16.0 ± 3.6*</td>
<td>62.1 ± 3.6</td>
<td>64.2 ± 3.7</td>
</tr>
</tbody>
</table>

p values * p < 0.05.
striking family histories for early morbidity and mortality from ischemic heart or cerebrovascular diseases. This suggests the possibility of an inherited impairment of their capacity to metabolize methionine. Homocysteine is the branch point between the transsulfuration and the transmethylation pathways. An accumulation of homocysteine can either be due to a reduced capacity of the enzyme cystathionine $\beta$-synthase or an abnormally low capacity to remethylate homocysteine to methionine by the folate and cobalamine or by the betaine dependent pathways.\(^1\)

Heterozygotes for cystathionine $\beta$-synthase deficiency have reduced ability to metabolize methionine (12.15) but have neither high fasting plasma MDS levels\(^2\) nor an increased frequency of heart attacks and strokes.\(^16\) Therefore it is unlikely that the carrier state for cystathionine $\beta$-synthase deficiency can explain the increased plasma MDS after an overnight fast in some of our patients.

Cystathionine $\beta$-synthase requires pyridoxal phosphate as a cofactor and homocysteinemia can be induced experimentally in rabbits by administration of 6-azauradine triacetate which causes pyridoxal phosphate deficiency.\(^17\) However, pyridoxal phosphate deficiency is rare but may cause homocysteinemia in humans as increased plasma homocysteine and thromboembolism are known complications in psoriatic patients treated with 6-azauradine triacetate.\(^18\) Pyridoxal phosphate deficiency does not appear to be a factor in our patients, but this possibility must be further investigated.

Abnormalities in the betaine dependent remethylation pathway that cause homocysteinemia are not known but the modification of a large number of varia-

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**FIGURE 2.** Plasma Cysteine-Homocysteine Mixed Disulfide (MDS) Concentrations after an overnight fast and 4 hours after a Methionine load (100 mg/kg) in control subjects and patients with arteriosclerotic cerebrovascular disease. Mean concentrations ± S.D. are shown. Values are higher in patients ($p < 0.05$) both before and after Methionine.

**FIGURE 3.** The relationship in control subjects and patients with arteriosclerotic cerebrovascular disease between plasma Cysteine-Homocysteine Mixed Disulfide (MDS) concentrations after an overnight fast and 4 hours after a Methionine load (100 mg/kg). $r = 0.718$, $p < 0.001$.

**FIGURE 4.** The relationship in control subjects and patients with arteriosclerotic cerebrovascular disease between Methionine dosages calculated as g/m$^2$ body surface area and plasma Cysteine-Homocysteine Mixed Disulfide (MDS) concentrations 4 hours after a Methionine load given as 100 mg/kg body weight. $r = -0.253$, $p > 0.05$. 
bles can affect the overall activity of the folate and cobalamin dependent remethylation pathway. One patient in the present study who had a serum cobalamine below normal also demonstrated the most elevated MDS concentration both before and after the methionine load. Low serum cobalamine may have contributed to the high MDS values in this patient due to a reduced capacity to remethylate homocysteine. The three control subjects with the highest MDS values before, mean 5.0 μmol/l, and after, mean 17.8 μmol/l, the methionine load received per orally 5mg folic acid daily for 4 weeks and were then reloaded. The therapy resulted in a substantial reduction of plasma MDS concentrations to a mean 3.0 μmol/l before and a mean 11.9 μmol/l after the load in all three subjects. All had serum folate an serum cobalamine within normal limits. Renal transplant recipients have increased plasma homocysteine concentrations that can be normalized by treatment with folic acid despite the absence of folate deficiency. In 1983 Wilcken et al reported identical twins with ischemic heart disease and increased plasma homocysteine both after an overnight fast and after a methionine load. Blood folates were normal but serum cobalamine were below or in the lower range. Oral folic acid restored homocysteine to normal. We therefore believe it likely that an abnormality in the folate and cobalamine dependent remethylation pathway is the cause for increased plasma MDS in some of our subjects. The results with folic acid therapy indicate the therapeutic possibilities if increased plasma homocysteine contributes to the pathogenesis of arteriosclerosis.

More detailed and extended studies are required to elucidate the exact role of homocysteine in the pathogenesis of arteriosclerosis and to what extent moderately persistent homocysteinemia contributes to morbidity in arteriosclerotic disease.

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