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

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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. Homocystinemia 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 homocystinemic 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 derivatives 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 ba-boons with homocysteine thiolactone caused patchy

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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 TIAs 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, ß-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 was 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 sulfosalicylic 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 weight, height 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 forlate 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 concentra-
tions between the groups. After the load there was
a more than a 20 fold increase in plasma methionine in
most subjects. No subject had a low post load methion-
ine concentration corresponding to poor absorption.

The mean plasma MDS concentrations were signifi-
cantly elevated both before (p < 0.05) and 4 h ours
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 corre-
lation 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 concentra-
tions were slightly elevated both before and after the
methionine load in patients and there was no signifi-
cant elevation of the concentrations after the load.

The correlation between the given dosages of meth-
ionine 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 correla-
tions (fig. 4). This discrepancy suggests that the given
dosage saturates one of the metabolic steps prior to
homocysteine in the transsulfuration pathway. No pat-
tern was found between received medications and the
mean pre and post load plasma MDS concentrations. This
discrepancy suggests that the given dosages had a low post load methionine concentration corresponding to poor absorption.

The two patients with the most elevated plasma
homocysteine measured as MDS both after an over-
night fast and after a methionine load in some patients
with arteriosclerotic cerebrovascular disease. As ho-
mocysteinemia is associated with premature arterio-
sclerosis and as experimental studies both jn vivo and
in vitro show that homocysteine causes endothelial cell
injury and cell detachment these data suggest that ho-
mocysteine might have contributed to the cerebrovas-
cular 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. Howev-
er, 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 pre-
vious study found no difference in MDS concentra-
tions between patients with chronic renal failure re-
ceiving and not receiving a variety of antihypertensive
agents and diuretics. Therefore the influence of med-
ications seems to be of minor importance but cannot be
excluded.

Wilcken et al 1983 could not reproduce their re-
results from 1976 in patients with ischemic heart dis-
ease. 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 sur-
fce 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

### Table 1: Mean Values ± S.E.M. of Relevant Data for Control Subjects and Patients with Arteriosclerotic Cerebrovascular Disease.

<table>
<thead>
<tr>
<th></th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Height (cm)</th>
<th>Body surface area (m²)</th>
<th>Serum cholesterol (mmol/l)</th>
<th>Serum triglycerides (mmol/l)</th>
<th>Serum folate (μmol/l)</th>
<th>Serum cobalamin (μmol/l)</th>
<th>Smokers (N)</th>
<th>Hypertension (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (N = 17)</td>
<td>47.5 ± 2.4</td>
<td>71.5 ± 3.4</td>
<td>173.3 ± 2.2</td>
<td>1.84 ± 0.05</td>
<td>5.2 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>11.8 ± 1.3</td>
<td>356.1 ± 33.3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Patients (N = 19)</td>
<td>49.3 ± 1.8</td>
<td>65.6 ± 3.0</td>
<td>168.2 ± 2.5</td>
<td>1.74 ± 0.05</td>
<td>5.5 ± 0.3</td>
<td>1.5 ± 0.3</td>
<td>13.3 ± 1.6</td>
<td>436.4 ± 57.2</td>
<td>14</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 metabolise 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 cobalamin or by the betaine dependent pathways.\textsuperscript{1}

Heterozygotes for cystathionine $\beta$-synthase deficiency have reduced ability to metabolise methionine (12.15) but have neither high fasting plasma MDS levels\textsuperscript{12} nor an increased frequency of heart attacks and strokes.\textsuperscript{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-azauraidine triacetate which causes pyridoxal phosphate deficiency.\textsuperscript{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-azauraidine triacetate.\textsuperscript{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-
biles can affect the overall activity of the folate and cobalamin-dependent remethylation pathway. One patient in the present study who had a serum cobalamin below normal also demonstrated the most elevated MDS concentration both before and after the methionine load. Low serum cobalamin 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 cobalamin within normal limits. Renal transplant recipients have increased plasma homocysteine concentrations that can be normalized by treatment with folic acid despite the absence of forlate deficiency. In 1983 Wilcken et al[12] 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 cobalamin 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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