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HIGH PREVALENCE OF COBALAMIN DEFICIENCY IN THE ELDERLY

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Hematologic and neuropsychiatric abnormalities caused by cobalamin deficiency (1). Cobalamin (Cbl, vitamin B_{12}) deficiency can cause a variety of hematologic abnormalities that include anemia, an increase in the erythrocyte mean cell volume, decreases in the white blood cell and platelet counts, and the presence of macroovalocytes and hypersegmentation of neutrophils on the peripheral blood smear. Examination of the bone marrow reveals hypercellularity of all elements and there is an abnormal cellular maturation with a distinctive morphology that is referred to as "megaloblastic." Erythrocyte precursors rupture within the bone marrow and release their contents which gives rise to increases in plasma levels of lactic dehydrogenase and bilirubin. Cobalamin deficiency also causes a variety of neuropsychiatric abnormalities that are due to patchy demyelinization in the posterior-lateral columns of the spinal cord, peripheral nerves, cerebral cortex and cranial nerves. Abnormal signs and symptoms develop which include abnormal proprioception, ataxia, paresthesias, irritability, memory loss, dementia, psychosis, and abnormal taste, smell and vision. Folate deficiency causes hematologic abnormalities that are indistinguishable from those seen in Cbl deficiency although it does not cause the neuropsychiatric abnormalities.

Serum Cbl levels in the elderly (2,3). For over 40 years, the serum Cbl assay has been the primary diagnostic test for Cbl deficiency. The normal range for serum Cbl is approximately 200–900 pg/ml and approximately 90% of patients with clinically confirmed Cbl deficiency (one or more hematologic or neuropsychiatric abnormalities of the type mentioned above that correct with Cbl therapy) have

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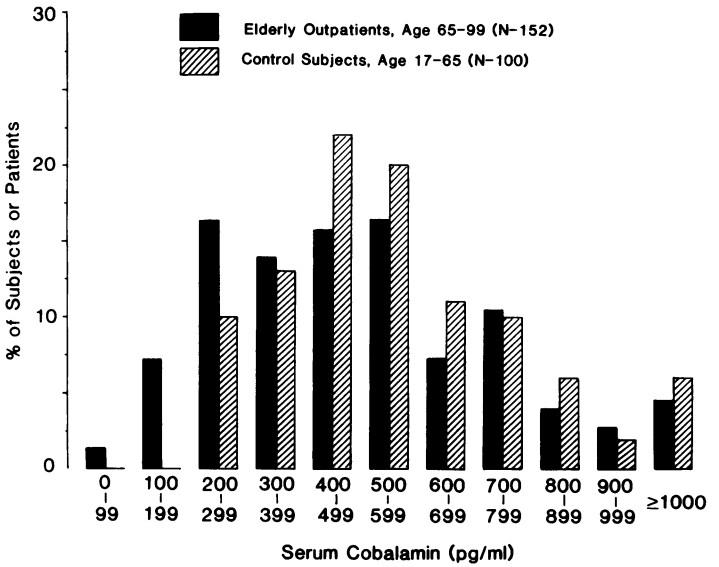


FIG. 1. The proportion of subjects with serum Cbl levels between 0 and 100, 101 and 200 etc. is shown for elderly outpatients, age 65–99 (N = 152) (solid bars) and the normal controls, age 17–65 (N = 100) (hatched bars). 10% of the normal controls and 16.4% of the elderly subjects had serum Cbl levels in the 200–299 pg/ml range (adapted from reference 2).

decreased levels of serum Cbl. By definition, 2.5% of normal subjects without hematologic or neuropsychiatric abnormalities have serum Cbl levels less than 200 pg/ml. Serum Cbl levels have been difficult to interpret in the elderly population since, as shown in Figure 1, there is a marked increase in the number of subjects with low (<200 pg/ml) and low normal (200–299 pg/ml) serum Cbl values. Because the vast majority of these elderly subjects lack the hematologic and neuropsychiatric abnormalities caused by Cbl deficiency, the shift toward lower Cbl levels in the elderly has been considered of little clinical significance.

Metabolic abnormalities in Cbl deficiency (4). Homocysteine and methylmalonic acid are metabolites that are related to the 2 mammalian Cbl-dcpendent enzymes as illustrated in Figures 2A and 2B. We have used gas chromatography/mass spectrometry to develop sensitive assays for these two metabolites. These assays have enabled us to quantitate the metabolites in normal sera and sera from patients with clinically confirmed Cbl and folate deficiency. As shown in Figure

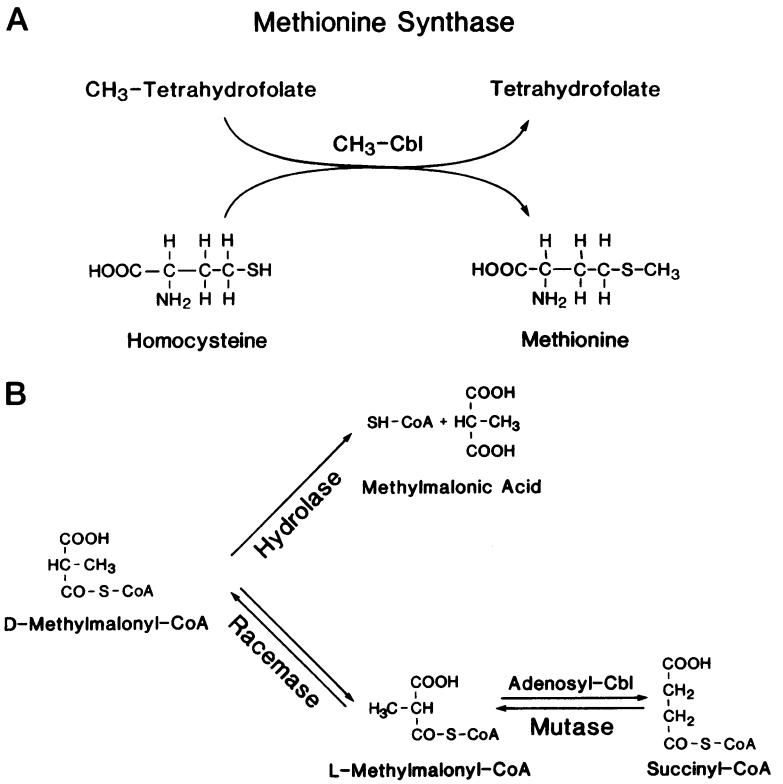
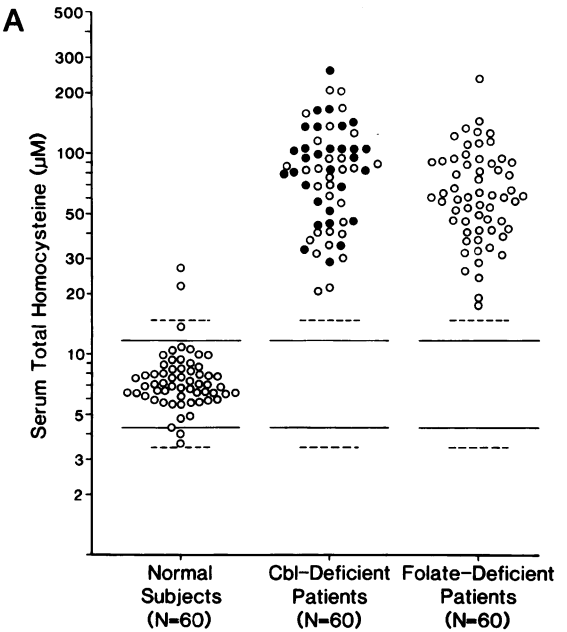


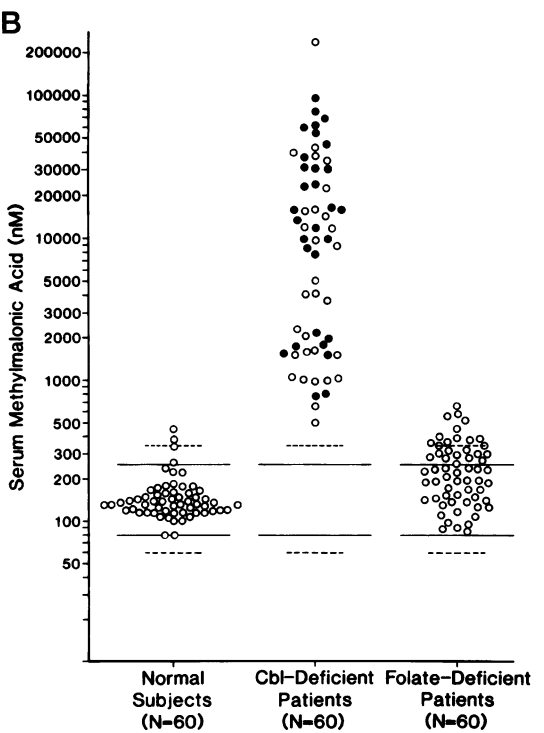
FIG. 2A. Reaction catalyzed by the CH_3-Cbl dependent enzyme, methionine synthase, in which a CH_3-group from CH_3-tetrahydrofolate is transferred to the SH-group of homocysteine to form tetrahydrofolate and methionine, respectively. Total homocysteine accumulates in both Cbl and folate deficiency.

FIG. 2B. Reactions involved in the metabolism of D-methylmalonyl-CoA L-methylmalonyl-CoA, and succinyl-CoA. In Cbl deficiency, L-methylmalonyl-CoA mutase is impaired due to a lack of adenosyl-Cbl. This leads to a buildup of L-methylmalonyl-CoA and D-methylmalonyl-CoA. Increased levels of D-methylmalonyl-CoA are hydrolyzed in increased amounts to methylmalonic acid by D-methylmalonyl-CoA hydrolase.

3A, total homocysteine[(a)] is elevated in virtually every patient with Cbl or folate deficiency Methylmalonic acid, on the other hand, is markedly elevated only in patients with Cbl deficiency. Some patients with folate deficiency have mild elevations of methylmalonic acid but this is due to concombinant dehydration or renal insufficiency. Elevated levels of

[a] The term total homocysteine refers to the sum of all forms of homocysteine present including that in homocystine (homocysteine-homocysteine disulfide), as well as homocysteine-cysteine mixed disulfide and that bound to cysteine residues in proteins and a variety of peptides.


A
Serum Total Homocysteine (μM)
500
300
200
100
50
30
20
10
5
3
2
Normal Subjects (N=60)
Cbl-Deficient Patients (N=60)
Folate-Deficient Patients (N=60)


B
Serum Methylmalonic Acid (nM)
200000
100000
50000
30000
20000
10000
5000
3000
2000
1000
500
300
200
100
50
Normal Subjects (N=60)
Cbl-Deficient Patients (N=60)
Folate-Deficient Patients (N=60)

total homocysteine and methylmalonic acid fall to normal when Cbl-deficient patients are treated with Cbl, but do not decrease if such patients are inappropriately treated with folic acid even in large doses. Levels of total homocysteine and methylmalonic acid remain elevated even in those Cbl-deficient patients whose hematologic abnormalities correct with folate therapy. The accurate diagnosis and appropriate treatment of Cbl deficiency is very important since neuropsychiatric abnormalities do not respond to folate therapy. Elevated total homocysteine levels due to folate deficiency do not respond to inappropriate therapy with Cbl. Thus, the demonstration of elevated levels of total homocysteine and methylmalonic acid that fall to normal with Cbl therapy provides convincing evidence for the existence of Cbl deficiency.

Metabolic abnormalities in the elderly (2,3). The availability of the new assays for total homocysteine and methylmalonic acid has enabled us to reevaluate the clinical significance of the shift toward low and low normal serum Cbl values in the elderly population that is depicted in Figure 1. Figures 4A and 4B illustrate the serum total homocysteine and methylmalonic acid levels, respectively, of the 10 of 100 controls and the 38 of 132 elderly subjects that had serum Cbl levels <300 pg/ml. Over half of the 38 elderly subjects had clear elevations of serum total homocysteine and/or methylmalonic acid that were often striking. In contrast, only a few of the 10 control subjects had elevations of either metabolite and these were very mild. Of the 114 elderly subjects with serum Cbl levels ≥300 pg/ml, 5 (3.5%) had an isolated elevation of total homocysteine, 7 (4.6%) had an isolated elevation of methylmalonic acid and 3 (2%) had elevations of both metabolites. Parenteral Cbl therapy of the patients with elevations of total homocysteine and/or methylmalonic acid with serum Cbl <300 pg/ml showed a marked decrease in both metabolites in every case. Similar decreases were also seen in the elderly subjects with serum Cbl levels ≥300 pg/ml who had elevations of methylmalonic acid with or without an elevated total homocysteine. Elderly subjects with isolated

FIG. 3A. Serum levels of total homocysteine in 60 normal subjects, 60 patients with clinically confirmed Cbl deficiency (30 without, ○ and 30 with, ●, neuropsychiatric abnormalities), and 60 patients with clinically confirmed folate deficiency. The solid and dashed horizontal lines represent the mean ± two and ± three SD, respectively, for the 60 normal subjects (adapted from reference 4).

FIG. 3B. Serum levels of methylmalonic acid in 60 normal subjects, 60 patients with clinically confirmed Cbl deficiency (30 without, ○, and 30 with, ●, neuropsychiatric abnormalities), and 60 patients with clinically confirmed folate deficiency. The solid and dashed horizontal lines represent the mean ± two and ± three SD, respectively, for the 60 normal subjects (adapted from reference 4).

TABLE 1
Incidence of Elevated Serum Total Homocysteine (tHcy) and Methylmalonic Acid (MMA) Levels in Various Elderly Populations

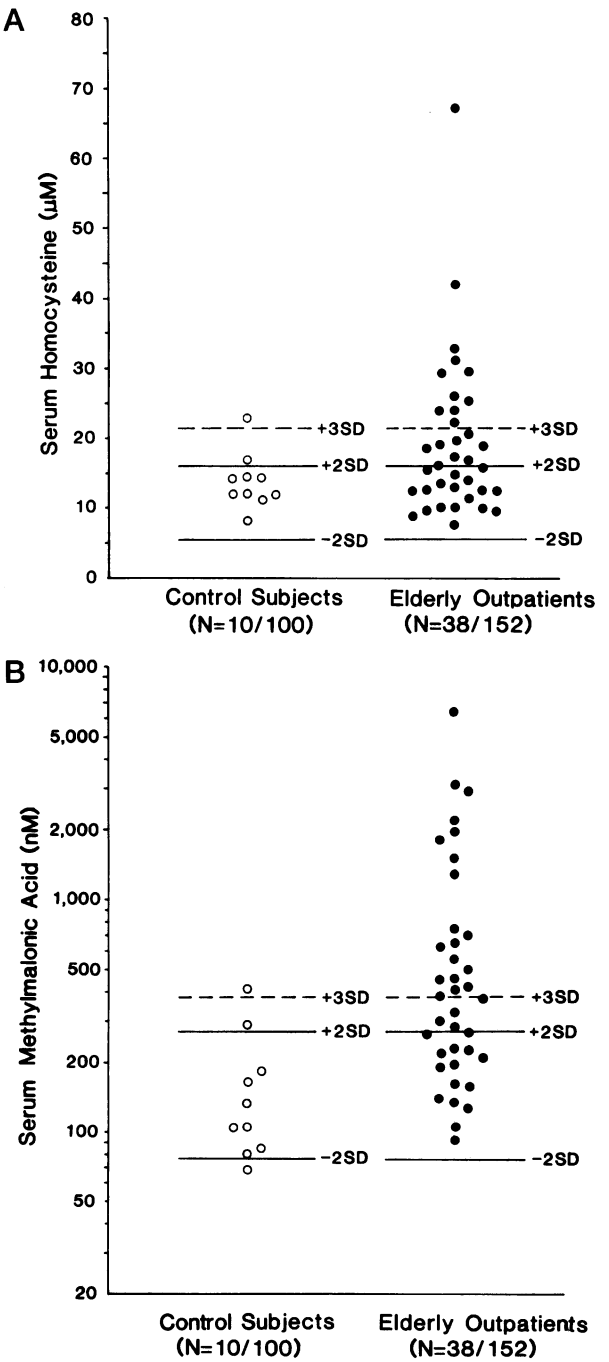
Group	Age		Number	Elevation of tHcvs and MMA		
	Mean	Range		tHcy	MMA	Both
	(yr)	*(yr)*		*(#) (%)*	*(#) (%)*	*(#) (%)*
Denver outpatients (2)	80	65–99	152	50 (33)	56 (37)	30 20)
Denver nursing homes[b]	84	55–99	212	47 (22)	72 (34)	28 (13)
Framingham initial cohort (3)	78	67–96	548	108 (20)	172 (31)	73 (13)
		Total	912	205 (22)	300 (33)	131 (14)

elevations of total homocysteine and serum Cbl ≥300 pg/ml were not studied. Only a few of the elderly subjects treated with Cbl showed significant hematologic and/or neuropsychiatric responses. Nevertheless, the incidence of elevated metabolite levels and their response to Cbl therapy demonstrate that the shift toward low and low normal serum Cbl levels in this elderly population is a reflection of definite although perhaps subclinical Cbl deficiency.

Table 1 summarizes the incidence of elevated total homocysteine and methylmalonic acid levels in the 152 elderly outpatients just described (2) together with 212 elderly subjects from a nursing home study and 548 elderly subjects from the initial Framingham cohort (3,4). The results are very similar in all three groups. Serum total homocysteine was elevated in 22% and serum methylmalonic acid was elevated in 33% of the combined total of 912 elderly subjects. Both metabolites were elevated in 14% of the elderly subjects which indicates that over 50% of the total homocysteine elevations were due to Cbl deficiency. Several additional studies with the initial Framingham cohort (5,6) suggest that folate and vitamin B_6 deficiencies are responsible for many of the isolated elevations of total homocysteine.

Serum total homocysteine and vascular disease. A large number of recent studies have shown that an elevation in serum total homocysteine is an independent graded risk factor for all forms of arteriosclerotic vascular disease (6–10) A recent meta-analysis of 27 such studies concluded that the odds ratio for all forms of vascular disease of a 5 μM increment in serum total homocysteine is approximately 1.5, and is equivalent to an increase in serum cholesterol of 20

[b] R. H. Hoffman, P. J. Shillam, M. Orleans, P. Archer, R. H. Allen, S. P. Stabler. The prevalence of B_{12} deficiency in a sample of Denver nursing home patients. In preparation, 1995.


A
Serum Homocysteine (μM)
80
70
60
50
40
30
20
10
0
+3SD
+2SD
-2SD
+3SD
+2SD
-2SD
Control Subjects
(N=10/100)
Elderly Outpatients
(N=38/152)
B
Serum Methylmalonic Acid (nM)
10,000
5,000
2,000
1,000
500
200
100
50
20
+3SD
+2SD
-2SD
+3SD
+2SD
-2SD
Control Subjects
(N=10/100)
Elderly Outpatients
(N=38/152)

mg/dL (11). Most of these studies, which involved patients who were less than 60 years old, indicate that a subclinical deficiency of folic acid is more prevalent than subclinical deficiencies of Cbl or vitamin B_6. Our studies (2,3) indicate, however, that subclinical Cbl deficiency is the most important deficiency in the elderly population although folate and vitamin B_6 deficiencies appear to play significant roles (5,6,12,13). This observation is important since the incidence of vascular disease is much higher in the elderly than it is in subjects less than age 60. Combined therapy with Cbl, folate and vitamin B_6 is particularly effective in lowering serum total homocysteine levels (13,14). Clinical trials have not been performed to demonstrate that an actual decrease in serum total homocysteine has a positive impact on the development of vascular disease. It is hoped, however, that such trials will be undertaken in the near future.

SUMMARY

In summary, we have shown that there is a high prevalence of Cbl deficiency in the elderly. This observation is based on an increase in the number of low and low normal serum Cbl levels, an increase in elevated serum total homocysteine levels that correct with Cbl therapy, and an increase in elevated serum methylmalonic acid levels that also correct with Cbl therapy. These observations may be of great clinical importance since Cbl deficiency may be a common and treatable cause of vascular disease.

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FIG. 4A. The serum total homocysteine levels for the control (age 17–65) (○) and elderly (age 65–99) (●) outpatients from Figure 1 with serum Cbl levels <300 pg/ml are shown. The normal range ±3 SD for serum total homocysteine is shown by the dashed lines and is 4–21.3 μmol/L. The normal range ±2 SD is shown by the solid lines and is 5–16 μmol/L (adapted from reference 2).

FIG. 4B. The serum methylmalonic acid levels for the controls (age 17–65) (○) and elderly outpatients from Figure 1 (age 65–99) (●), with serum Cbl levels <300 pg/ml are shown. The normal range ±3 SD for serum methylmalonic acid is shown by the dashed lines and is 43–376 nmol/L. The normal range ±2 SD is shown by the solid lines and is 73–271 nmol/L (adapted from reference 2).

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DISCUSSION

James Cerda, Gainesville: Is there a question of bacterial over growth?

Allen: In over 2000 patients, half of whom have had elevated metabolites, we have shown that we could decrease the metabolites with appropriate vitamin therapy, but we have not looked in detail at what the etiology of the lower cobalamin levels and the elevated metabolites are.

Cerda: Might it all go away with antiinfective therapy?

Allen: That is possible, in some patients.

Fred Schiffman, Providence: Is there perhaps any connection in your methionine hypothesis with the association between the neurologic effects that occur and the anemia that occurs? Dr. Lindenbaum and others have shown this association in groups of patients. Do you think this pathway may be involved in that?

Allen: It is still a mystery as to what causes the neuropsychiatric abnormalities in

cobalamin deficiency. I think it is clear that methionine synthase impairment is responsible for the hematologic problems, since that involves both cobalamin and folate. Whether it is the mutase or the methionine synthase is not known. There is something missing in our understanding. We have looked at a number of patients and were unable to find a metabolic correlation.

Palmer Futcher, Baltimore: Dr. Allen, classical cases of pernicious anemia can have gastrointestinal symptoms. Do you have any information whether these older patients with low cobalamin levels have increased gastrointestinal symptoms such as diarrhea, abdominal pain, increased flatus?

Allen: I am not aware of any information to suggest that and a number of the studies with information of that kind are available. What is so remarkable is that the incidence of subclinical cobalamin deficiency in the elderly is of the order of 15–20%. If you go out and get people at random, especially people that are not in the hospital, people that may be attending a geriatric clinic, or seeing their physician every six months or so, certainly 15–20% of them do not have significant gastrointestinal complaints or neurologic problems or anemia. It is as if it is a subclinical deficiency. In many cases we have serum samples from John Lindebaum's collection in which we can go back 10–15 years and these elevated metabolites and lowish vitamin levels seem very stable. It is not like pernicious anemia wherein once the disease starts it progresses and patients often die within six months or a year. It is subclinical and if the tie-in with homocysteine and vascular disease holds up, then I think we have to say it is a real disease and it is not just a subclinical curiosity.

Stephen Goldfinger, Boston: I do agree with you about the 90% likelihood that this will be of fundamental value in our management of cardiovascular disease from the standpoint of prevention. I would just like to make a comment on the side. This is a wonderful example of clinical epidemiology, not only providing us valuable information, but also giving credibility to the life's work of somebody a few of us would have known when he was a resident in pathology at Massachusetts General Hospital. I refer to Dr. Kilmer McCully, whose professional career suffered considerably by his commitment to the homocysteine story. Only lately has he been given the recognition he justly deserves. Some of you may remember quite a nice article about him and his life's work on this in the N.Y. Times.

Allen: Yes, I agree with his contribution. I tried to make it clear that he is the one back in 1969 who saw the second inherited disease that caused an elevated homocysteine, and the other metabolites that were abnormal were in the opposite direction. He then put forth the hypothesis that homocysteine in some way had something to do with vascular disease. It looks like he is going to be correct, not just in these very rare and unusual patients, but in a major segment of our population.

Peter Harpel, New York: Many of us have been impressed, particularly by the JAMA article on the linear relationship between homocysteine and vascular disease. There doesn't seem to be a threshold; so it makes sense that if you lower homocysteine, no matter what your level is, that it might have great benefit. A lot of us have been impressed that a relatively small amount of folate will lower homocysteine 50%: 400 micrograms, which is that amount of folate found in many multivitamins, will do it. So a lot of us are taking multi-vitamins and have suggested to our patients that they do it. In light of your new findings with cobalamin deficiency in the elderly, do you think this is hazardous?

Allen: In the JAMA article, and a number of articles that do meta-analysis on the vascular disease studies, most studies are done in people under age 60 and they are done in that group because you don't want to complicate things with a large number of additional illnesses. There is no question that under age 60 folate is most effective in

lowering the homocysteine, although cobalamin therapy also causes a significant decrease. Vitamin B6 usually does not. However, if you try to extrapolate these findings to the elderly, my point is you can't. I'm not saying folate is not important, but I think cobalamin is at least as important and maybe more so and I think B6 is also important. Without other studies at least, I think it would be a big mistake to just start treating everyone with additional folate, particularly since some of these patients will have pernicious anemia and you'll mask the neurologic problems.

Tony Gotto, Houston: Dr. Harpel is going to talk about lipoprotein tomorrow. Some in vitro data suggest that Lp(a) and homocysteine enhance the toxicity of one another. Harpel et al. found that homocysteine, at concentrations as low as 8 microM, significantly increased the affinity of Lp(a) for fibrin [Proc Natl Acad Sci 1992;89:10193–7]. Do you have any data, or are there any from Framingham, about Lp concentrations in association with elevations of homocysteine and increased vascular risk?

Allen: Peter is going to talk about some of that tomorrow. I am not sure of any direct data. It is clear though that homocysteine has been looked at by eminent statisticians. It is clearly an independent risk factor, but of course, it is additive to other things such as an elevated cholesterol, smoking, high blood pressure and any abnormalities of Lp(a) that would predispose to vascular disease.

Orville Horwitz, Philadelphia: I just want to ask one simple question. Is there any evidence that heparin therapy changes the homocysteine level?

Allen: Not that I am aware of. I don't know that this has been looked at.

George Schreiner, Washington: Back in the 60's and the time frame you were mentioning, Charlie Rath was studying various time courses of hematologic responses to B12 and folate and needed some additional patients and wanted some of my kidney patients with normal levels of kidney functions. We had a couple of outliers in that group that had enormous responses, almost to the point of looking like pernicious anemia. They turned out to have pyelonephritis; so I would like to stress two things. One, in elderly males who have a high incidence of prostatic obstruction and infection, this is still a common cause of deterioration of kidney function. Don't forget that sclerosis in the kidney occurs along with sclerosis in the coronaries and in the cerebral vessels and is probably also a manifestation of aging.

Allen: Although vitamin deficiencies appear to be the most common cause of an elevated homocysteine, renal impairment is also a common cause and it takes a very modest decrease in creatinine clearance to increase the homocysteine. Vascular deterioration is a very common phenomenon in all forms of renal disease. We can't normalize homocysteine in these patients. We can lower it. It would be interesting to see if we could have an effect on the progression of renal disease.