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Usefulness of the Co-Twin Control Design in Investigations as Exemplified in a Study of Effects of Ascorbic Acid on Laboratory Test Results

To the Editor:

There have been many investigations on the effects of drugs, diet, or other exogenous factors on biochemical variables. Such investigations require either that many subjects be allocated to control and treatment groups or that each subject act as his own control, with temporal separation of the treatment and control periods. The latter approach is the more economical in most cases, but long-term variability, either biological or analytical, can produce effects not truly ascribable to the factor under investigation.

Christian and Kang (*1*) showed that studies involving monozygotic ("identical") twins have considerable advantages. In this design one twin receives the "treatment" and the other a placebo (or nothing), and it can be shown that the relative efficiency of this design as compared with use of randomly selected individuals for the two groups is approximately equal to the ratio of the within-group to within-pair variances.

Several workers have reported that vitamin C, taken in the large doses that are currently popular, may affect results of laboratory tests (*2, 3*). Most of these reported effects have been ascribed to interferences with methods, but true effects on plasma lipids, which could be of some significance, have been both claimed and denied (*4*). It has also been claimed that the effects are most marked in those with hypercholesterolemia. We used the co-twin control method to investigate the medium-term effect of vitamin C on 23 biochemical tests.

One hundred and six pairs of monozygotic twins, ages between 14 and 65 years, participated in a trial of the effect of vitamin C on cold symptoms. One twin from each pair took 1000 mg of vitamin C daily and a multivitamin tablet that included 70 mg of vitamin C. The other twin took the multivitamin tablet (to exclude effects due to vitamin deficiency) and placebo tablet. The trial extended over 100 days, from late autumn to late winter.

Before and at the end of the trial, blood was taken for biochemical analysis. Plasma creatine kinase (EC 2.7.3.2) and lactate dehydrogenase (EC 1.1.1.27) were measured in a Centrifichem 300 centrifugal analyzer, with use of Calbiochem "Statpack" reagents; high-density lipoprotein (HDL) cholesterol by the method of Allen et al. (*5*); and other analytes by continuous-flow analysis in a Technicon SMAC (cholesterol by cholesterol oxidase, EC 1.1.3.6).

Table 1 shows the relative efficiencies of this design for a group this size. "Relative efficiency" may be interpreted as the increase in the number of subjects required, if they were drawn at random (unpaired) from this population, to attain the same significance levels. Table 2 shows the tests for which a significant difference between treatment and placebo was found, and the results for the lipid analyses. For the remainder of the tests, either no significant change occurred or the change was the same in both groups.

If one selects only the 16 pairs with a mean initial cholesterol above 6.5 mmol/L, the vitamin C group cholesterol fell by 0.69 mmol/L, the placebo group cholesterol by 0.44 mmol/L; the difference between them was not significant ($p = 0.21$), but this may merely reflect the small number of pairs available in this group.

The ratios of variances—and hence the relative power of the co-twin control design—were quite high (2.29 to 7.35). These values reflect the similarity between pairs of monozygotic twins, due both to their common genes and their shared environments. For the analytes of most interest, the lipids, the relative efficiencies were between 2 and 3.

Most of the results were not affected by vitamin C in a dose of 1070 mg a day, as compared with the placebo regime of 70 mg a day. In the two cases where significant effects were found, alanine aminotransferase (EC 2.6.1.2) and uric acid, the change would not be sufficient to cause difficulty in test interpretation. The tests where a true effect would be of greatest pathological significance are those for cholesterol and its fractions and for triglycerides. No statistically significant effects were found, even though it can be calculated that there was a 95% chance (β) of detecting at the 0.05 level (two-tail, α) a difference between groups of 0.17 mmol/L for cholesterol, 0.25 mmol/L for triglyceride, or 0.06 mmol/L for HDL cholesterol.

Evidently the co-twin control method is an efficient experimental design for studies such as this, and would be especially useful where the analyses required are expensive or difficult. Moreover, vitamin C in doses of about 1000 mg a day has no important effects on these test results when compared with a placebo regime providing adequate but not excessive vitamin intakes. Higher doses might have an effect, and acute effects might occur that are only temporary and revert to normal within three months.

Table 1. Relative Efficiency of Trial Designs

Alanine aminotransferase	2.89	Glucose	7.35
Albumin	2.48	HDL cholesterol	2.60
Alkaline phosphatase	5.84	Iron	3.13
Aspartate aminotransferase	2.29	Lactate dehydrogenase	3.83
Bicarbonate	5.44	Phosphate	4.00
Bilirubin	3.72	Potassium	5.66
Calcium	4.80	Protein	3.60
Chloride	4.14	Sodium	5.35
Cholesterol	2.88	Triglycerides	2.77
Creatine kinase	2.76	Urea	2.37
Creatinine	4.33	Uric acid	4.00
γ-Glutamyltransferase	2.30		

Table 2. Changes on Vitamin C and Placebo Regimes

Test	Mean change: Vit. C	Mean change: Placebo	p
Alanine aminotransferase, U/L	7.47	4.12	0.017
Uric acid, mmol/L	−0.006	0.005	0.038
Cholesterol, mmol/L	−0.29	−0.15	0.054
HDL cholesterol, mmol/L	−0.03	0.01	0.085
Non-HDL cholesterol, mmol/L	−0.26	−0.16	0.068
Triglyceride, mmol/L	−0.13	−0.02	0.16

Note: Negative value indicates decrease from initial reading.

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