Letters

Use of Twins in Epidemiological Research

The work of Osborne and Adlersberg on serum lipids in adult twins [*Science* **127**, 1294 (1958)] seems to us to be important, both because of the findings and because of the epidemiological method employed. We have therefore reviewed critically the experimental design and the results obtained.

One factor which is hard to assess in this study is the extent to which the sample is biased as a result of the difficulty of obtaining pairs of twins. It is clearly too much to expect that the subjects used will be selected at random from the target populations; the best one can do is to take available subjects, recognize the major sources of bias, and draw conclusions that are subject to the serious limitations imposed by these biases. For example, since the level of serum lipids changes with age [D. Adlersberg, *J. Am. Med. Assoc.* **162**, 619 (1956)], one would be cautious in making comparisons between any groups that differed in the distribution of ages. As some of the groups in this investigation were small and the age of the subjects ranged from 18 to 55 years, it may well be that age differences influenced the results obtained. Further, one would expect that, as age increased, twins would be more likely to live apart. If this occurred, the effect of environmental differences due to living apart would be partially confounded with differences due to age.

The authors classified twins into five main groups according to sex and zygosity; monozygous male, monozygous female, dizygous male, dizygous female, and dizygous of unlike sex. The numbers belonging to these groups at birth are approximately in the ratio of 1:1:1:1:2 [F. Sandon, *J. Roy Statist. Soc.* **120A**, 440 (1957)]. It is not essential that the population proportions should be preserved in the sample, provided that the sampling is representative, in each case, of the appropriate group. However, when, as in this instance, the proportions are changed considerably (the dizygous male group and the dizygous group of unlike sex, in the sample, are relatively small) and random sampling has not been explicitly applied, one becomes suspicious that selective factors might be operating. The same kind of point could be made by noting that the monozygous pairs outnumber the dizygous by 43 to 39, that female pairs are more numerous than males in the ratio of 46 to 27, and that the number of female pairs living together is the same as the number living apart; in each case the deviation from the corresponding population ratios is considerable.

Each of the five groups of twins described above was subdivided according to whether the twins lived together or apart. It seems likely that twins living apart would be relatively hard to enlist as subjects, and that those living apart who came into the sample might therefore be unrepresentative of their appropriate groups. The authors note that they obtained only two pairs of dizygous male twins living apart. In our experience, twins living together are likely to appear at the laboratory together, and thus to have blood drawn at the same time of day, under similar circumstances, and to have it analyzed in the same batches. When this happens, important sources of intrapair variance are controlled, and the data are not strictly comparable with data obtained in the absence of such controls.

A point in the analysis of the data calls for comment. In computing an interpair variance for 14 pairs of monozygous male twins living in the same house and for 5 pairs living apart, the authors used a common mean for the 19 pair averages and thus obtained 18 degrees of freedom for a pooled interpair variance. This procedure is biased in the direction of increasing the interpair variance in those cases where there is a difference between the means of the "together" and "apart" groups. Indeed, the conventional method of detecting a difference between the means of twins living together and twins living apart would be to test for nonrandom enlargement of the interpair variance as calculated by the authors. This potential bias resulting from differences in the group means is avoided if interpair variances are computed separately for the two groups, and subsequently pooled (unless they are significantly different).

The authors are to be congratulated on their attempt to develop a new approach to an important epidemiological problem. Publication of their full data, including the ages of the subjects, may resolve some of the problems discussed above. It does seem, however, that there are special difficulties in studying, in this way, an age-dependent variable such as serum cholesterol. These difficulties are not so acute when the variable under consideration is something like blood group or intelligence quotient, which does not vary with age.

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We appreciate the interest of White, Zalokar, and Pilot in our paper ["Serum lipids in adult twins," *Science* **127**, 1294 (1958)] and the opportunity of adding

a few remarks which could not be presented in a brief report.

It is well established that serum cholesterol and serum phospholipid levels are sex- and age-dependent variables. From previous studies [Adlersberg *et al.*, *J. Am. Med. Assoc.* **162**, 619 (1956)], it is known that in males there occurs a significant increase of total cholesterol and phospholipids in serum from age 20 to age 33 years. In females, however, these levels do not change significantly from age 2 to 32 years, but a significant rate of increase occurs from age 33 to 58. Although the ages of the subjects ranged from 18 to 55 years in our sample, the majority fell into the third and early fourth decades. The age differences between the two groups compared were small, for example, in monozygotic male twins, those living together had a mean age of 25.04 and those living apart, of 28.20, a difference of only three years. The greatest age difference was encountered in female dizygotic twins; those living together had a mean age of 20.56 and those living apart, of 33.23. In these age periods, serum lipids are not an age-conditioned variable.

We sympathize with the difficulties apparently experienced by White and his coworkers in obtaining twins living apart for simultaneous study. Because of the extreme importance of this precaution, the simultaneous physical and chemical examination of the two members of a twin pair was made a *conditio sine qua non* in our study. Rigid application of this principle often required many months of negotiation with the twin subjects. In addition, all specimens obtained were labeled by number, and their identity remained unknown to the laboratory personnel. Thus, any extrinsic effects upon intrapair variances were reduced to the practicable minimum.

In any epidemiologic study of man, sampling poses one of the most difficult problems. In the sampling of adult twins in good general health, the incidence of sex and zygosity at birth is of limited value. Greulich concluded, as early as 1934, that the number of twins in the general adult population was approximately 50 percent of the incidence at birth [*Am. J. Phys. Anthropol.* **19**, 391 (1934)]. It is now well established that the sex and zygosity differential of twins in adulthood is subject during life to marked modifications. (The sampling of the twin population under study is discussed in detail in a monograph by Osborne and DeGeorge, now in preparation).

We fully agree with White *et al.* on the method of analysis to be used, and what they suggest had, in fact, been done. In our study the average lipid levels in the various groups were calculated, compared, and found not to differ significantly. Interpair variances were calculated separately and found not to differ significantly, and subsequently they were pooled.

The method of study, as well as the evaluation of the data, is discussed in greater detail in our paper entitled "Serum lipids, heredity, and environment: A study of adult twins [*Am. J. Med.*, in press].

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Meetings

American Council on Education

The response of education in the United States to the sputniks and the challenges of the space age will be discussed by Arthur S. Flemming, Secretary of Health, Education, and Welfare, and other national leaders in education at the 41st annual meeting of the American Council on Education in Chicago, 9–10 October. The general theme, "Education Accepts New Challenges," will