utes to the measured turbidity, and the value of 1.36 is therefore bound to exceed the true background value. The four other Antarctic stations were located at elevations from sea level to 3000 feet (914 m), with no correlation between elevation and the ratio. These data thus suggest that altitude is not a factor.

Despite the probable increase in the atmospheric aerosol load, it is still possible today (as Porch *et al.* noted) to observe values of the ratio of $b_{scat}/b_{Rayleigh}$ very close to unity at remote stations. There is thus evidence of a high variability of aerosol loading at the low end of the scale, just as there is frequent evidence of extreme variability at the high end of the scale at polluted urban stations.

In sum, it appears that an atmospheric aerosol background level exists and that it is increasing.

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Acetylsalicylic Acid and Chromosome Damage

Mauer *et al.* (*1*) state: "No significant elevation of simple chromosome aberrations (breakage) was observed at any of the concentrations of ASA [acetylsalicylic acid; aspirin] tested. . . ." Their table 1 provides a list of ASA effects from 72-hour exposure at concentrations of 0, 1.0, 12.5, 25.0, and 50.0 μg/ml; the actual number of breaks they observed may be calculated. After combining data from all subjects, the concentration at 12.5 μg/ml produces a significant increase in chromosome breaks (11/425 versus 18/1453 in controls; $t = 2.13$; $P < .05$). The data at this concentration are not homogeneous; subjects should be compared with their own controls. At this concentration only, subject M.L. had significantly more breaks than his baseline (4/108 versus 3/397; $t = 2.41$; $P < .05$) as did subject I.M. (4/104 versus 4/411; $t = 2.20$, $P < .05$). Thus two of three subjects showed significantly elevated chromosome breaks at 12.5 μg/ml, and Mauer's conclusion stated above is not wholly valid.

The clear discrepancy between their results and those of Jarvik and Kato (*2*) remains, and perhaps should be resolved. I examined the effects of ASA in an unpublished pilot study. There was a significant fourfold increase in simple chromatid gaps at 6.5 μg/ml (17/107 versus 4/100; $t = 3.01$, $P < .01$). There was no increase in simple chromatid breaks, but two different chromosome-type aberrations were seen in treated cultures and none in controls. I believe this procedure not fully justified; but conversion of these to an "equivalent" number of breaks and adding raises the "break" frequency to significance (8/107 versus 1/100; $t = 2.30$, $P < .05$). Higher and lower concentrations gave no evidence of significant effect. It seems to me that a suspicion remains, albeit a small one; but I am inclined to agree with Mauer *et al.* that ASA probably is not much of a cytogenetic hazard.

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In their report Mauer *et al.* (*1*) state that ". . . in the only cytogenetic investigation which, to the best of our knowledge, exists in the world literature, Jarvik and Kato . . . reported an average breakage frequency twice the control value . . . in normal human leukocyte cultures treated with 0.1 and 1.0 μg of ASA [acetylsalicylic acid] per milliliter of medium 4 hours before harvesting of cells. . . . We have been unable to confirm these results" (*1*, p. 199). A look at table 1 of their own report, however, reveals that in the three persons for whom the experimental conditions described by Jarvik and Kato (*2*) were duplicated (donors M.L., L.K., I.M.), the results of Jarvik and Kato were replicated remarkably well. When no ASA was added (first line) there were eight breaks in the 1200 metaphases counted, or 0.67 percent. When ASA (either 0.1 or 1.0 μg/ml) was added, there were 12 breaks in 643 metaphases counted, or 1.87 percent. Thus, there was the doubling described by Jarvik and Kato.

The test used by Mauer *et al.* (*1*) for the statistical significance of the differences between the experimental and the control cultures is an insensitive one. They set 95 percent confidence limits on each proportion of broken chromosomes, found that the intervals almost invariably overlapped one another, and concluded that no significant differences existed.

One defect in this procedure is that the significance level for each comparison between an experimental culture and the control culture is necessarily less than the apparent 5 percent level (how much less depends on the proportions involved), making it unduly difficult to detect significant differences. The second defect is that no incorporation is made in the analysis of the consistency, if any, of the differences from one subject to the next.

A summary chi-square test due to Cochran (*3*) overcomes both of these defects. It keeps the significance level at .05, say, the desired probability, and incorporates any possible consistency of differences across subjects. Applying Cochran's test to the data of Mauer *et al.*, it is found that, even though none of the chi-squares for the three individual subjects is significant, nevertheless the summary chi-square, also with 1 degree of freedom, is significant ($\chi^2 = 5.15$, $P < .025$). The reason is that the individual differences, although not large enough to attain significance by themselves, are all in the same direction and of about the same magnitude.

Several investigators using a number of drugs, especially LSD, have failed to demonstrate a dose-related increase in breaks [see review by Moorhead *et al.* (*4*)]. With regard to ASA, Jarvik *et al.* (5, p. 1251) stated ". . . (0.1 and 1.0 mcg/ml–dosages were combined since the frequency of breaks was similar in both). . . ." Among the possible reasons for this apparent plateau may be a selective susceptibility of damaged cells to the very drug that kills them, yielding a spuriously low relative frequency of breaks at higher doses. Drugs such as streptonigrin may exhibit a more limited spectrum of toxicity than ASA and thus damaged cells might survive more readily in the presence of the former drug than in the presence of the latter.

In a further experiment Mauer and associates could not demonstrate that ingestion of ASA had an effect on frequency of chromosome breakage. Failure to detect an in vivo response could be due to many reasons, among them sequestration of damaged cells, mitotic inhibition, or the fact that the life-span of the human lymphocyte is reckoned in months and years, rather than days or weeks. True lack of damage is not the only explanation.

Finally, while Meisner *et al.* (*6*) indeed "reported no *dose-related* [italics ours] chromatid breakage after the addition of ASA to continuous cultures of human skin," Mauer and colleagues (*1*, p. 200) failed to add that Meisner and associates did find a *threefold increase* in chromatid breaks occurring 1 week after treatment with 100 μg/ml and 200 μg/ml and, even more important, that they found evidence of chromosome exchange and rearrangement (quadriradials, dicentrics, fragments, and abnormal chromosomes). While the significance of simple chromatid breaks is still unknown, most investigators agree that exchanges and rearrangements are indicative of mutagenesis.

To paraphrase Galileo, "Eppur si rompe"; it appears that in vitro aspirin does break human chromosomes after all.

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We are in a state of flux with respect to testing for the significance of chromosome damage, as both Loughman's and Jarvik and Fleiss's comments attest (*1*). All contributions and debates should lead us to more dependable methods. In the meantime, all we have working for us is the sharing of our findings in as detailed a manner as possible, recognizing that equivocal results with the human leukocyte test system require in the last analysis much more work combined with sharper, but at the same time more realistic, statistical methodology.

In our statement (*2*) that we could not confirm the results of Jarvik and Kato with aspirin in vitro (*3*), we were indeed referring to our inability to detect any increase in chromosome damage with increasing concentrations of the drug. The rationale advanced by Jarvik and Fleiss (*1*) for the failure to demonstrate such a dose-related increase in breaks in vitro ("selective susceptibility of damaged cells to the very drug that kills them, yielding a spuriously low relative frequency of breaks at higher doses") is extremely interesting. We await definitive experimental verification of this hypothesis. In the absence of such verification, we cannot understand why a substantial increase in damage per cell (such as simple breaks) is not detectable after treatment with aspirin before overt necrosis occurs (for example, 50 percent reduction in mitotic index); such damage continues to result from our experiments with caffeine, in which case we can detect treated cells with as many as ten breaks. If all denominators were equal (and infinitely large!), the analysis would be obvious. As they are not, we chose the method of Stevens (*4*) for mutation rate over other statistical tests, which are fine if the parameter were survival or death of a guinea pig. Thus, for the labile biological parameter we are attempting to assess (that is, borderline gaps versus breaks), mere arithmetic doubling falls in the realm of pure chance by our statistical analysis.

In our study we were attempting to amplify (be it confirm or not) this oft-cited experiment (*3*) that has been referred to as evidence that "Several commonly administered agents such as aspirin . . . are also reported to cause a *striking* [italics ours] increase in chromosome breakage" (*5*). Further, since we agree with Jarvik *et al.* (*6*) that "Clearly in vitro findings cannot be applied in vivo without careful further study," we did test aspirin in vivo, with the stated negative results. We also agree (as we have stated) that since we did not sample any other tissue but lymphocytes we could never be sure chromosome damage does not occur in other tissues, for which "sequestration of damaged cells" as well as "mitotic inhibition" (*1*) might be advanced as hypotheses for nondetection. Neither of these "reasons," however, can apply in our in vivo study, since respectable (and uninhibited, according to our standards) mitotic cell populations were obtained throughout the period of study (5 weeks), and hence we cannot envisage such sequestrations of damaged cells which would not be reflected in suspiciously low mitotic indices during some portion of that treatment period. We also fail to see how "the life-span of the human lymphocyte" undisturbed in situ (be it months or years) is relevant to a critique of our guarded conclusions, since a sample from that circulating population of cells withdrawn and induced to divide by phytohemagglutinin did not manifest cytogenetic damage in our hands.

Finally, we were not begging confirmation from the necessarily incomplete abstract of an oral presentation given by Meisner and associates at the 1969 conference on somatic cell genetics (*7*), but accepting the senior author's own critical assessment of the significance of their results after addition of aspirin to continuous cultures of human skin. It would be best to await publication of the full study for further comment.

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