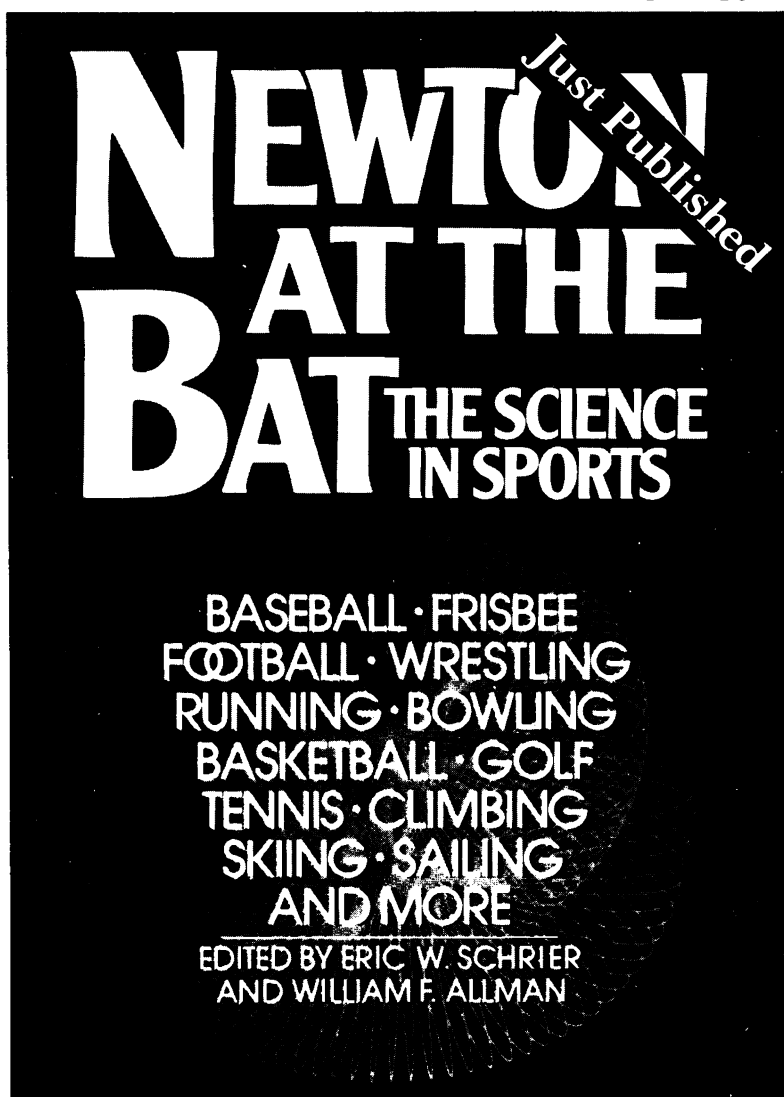


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LETTERS

Bacterial Contamination of Human Tumor Samples

Since the discovery that certain human tumors contain activated oncogenes, a great deal of effort has been dedicated to searching for genetic alterations that may explain their neoplastic properties. While screening human carcinomas for possible rearrangements within *ras* oncogenes, we have encountered an artifact that I would like to report to other investigators engaged in similar endeavors. We have detected significant amounts of pBR322-related sequences, presumably of bacterial origin, in DNA's isolated from about 20 percent of colonic tissues (both tumors and polyps) and occasionally in tumor material that was stored frozen in a repository. We suspect some of the latter material may have been suboptimally stored. These human tumor DNA's contain DNA fragments of a high molecular weight (8 to 30 kilobase pairs) that hybridized with certain oncogene probe preparations. This hybridization was due to a low percentage of pBR322 sequences which are invariably present in probes obtained from DNA fragments subcloned in pBR322, even after two cycles of electrophoretic purification. Because the relative contribution of these residual vector sequences varies tremendously with the size of the DNA fragments used as probes, the pBR322-related DNA fragments present in human tumor DNA were detected with probes of less than 300 base pairs but not with probes of 2 kilobase pairs or larger. In view of these observations, I strongly recommend the routine use of vector probes as controls in Southern blot analysis of human tumor DNA's.

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Recently in the study of hepatitis B virus (HBV) integration and hepatocarcinogenesis, there has been concern about getting false positive results due to bacterial contamination of autopsy materials.

Using both an HBV DNA probe and a pBR322 probe, we hybridized DNA from hepatocellular carcinomas (HCC) obtained at autopsy from 70 humans. The HBV DNA probe showed 1 to 6 bands per case in 32 HCC's, whereas the pBR322 probe revealed bands in 15 HCC's, including a hepatoblastoma,

with exactly the same pattern as that obtained with the HBV DNA probe. Histological study revealed bacterial colonies in some HCC tissues that had given rise to the bands with the pBR322 probe. This indicates that false positive bands may result from hybridization of the probe with bacterial plasmids in autopsy materials.

When these false positive cases were deleted, our corrected figures for the integration of HBV DNA were as follows: 15 out of 20 as opposed to 18 out of 20 HCC's from carrier patients; 0 out of 16 as opposed to 6 out of 16 HCC's from patients with HBV antibodies (noncarriers); and 1 out of 34 as opposed to 4 out of 34 HCC's from patients clinically negative for HBV.

OKIO HINO
TOMOYUKI KITAGAWA
HARUO SUGANO

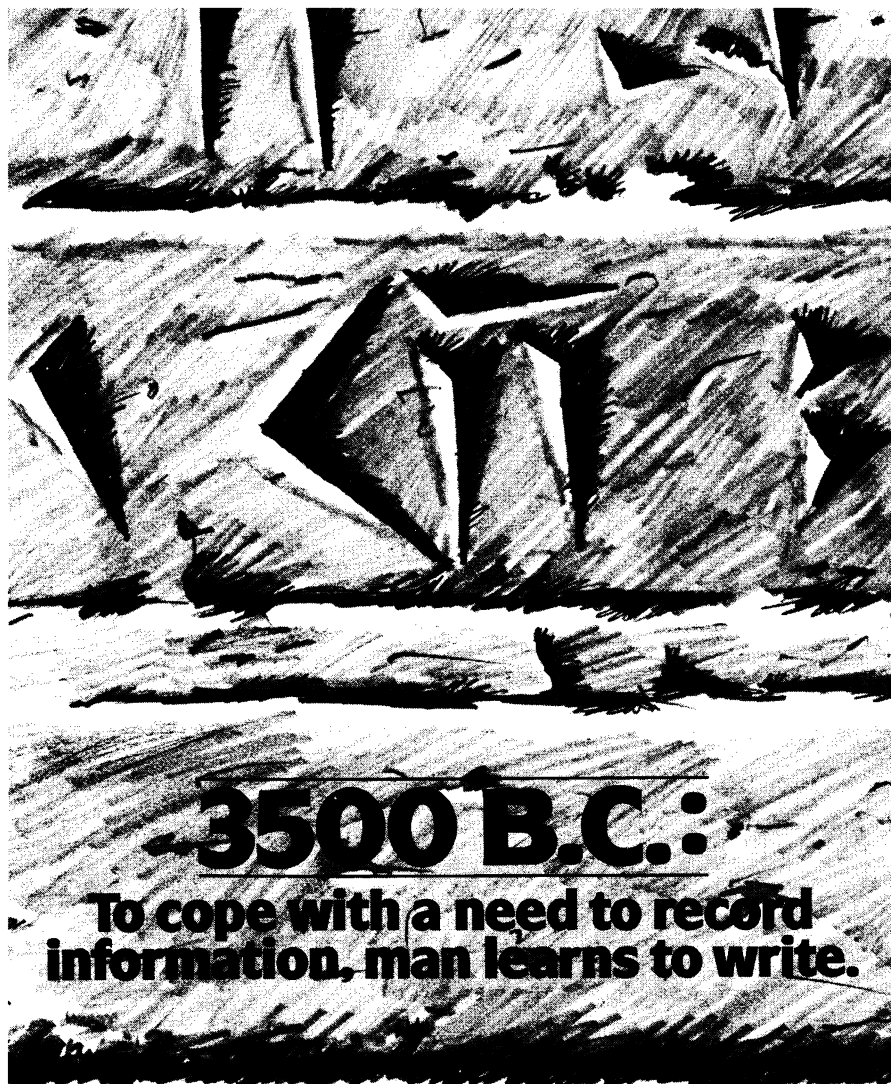
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Seroepidemiology

Gina Kolata, in her commentary on "The new epidemiology" (News and Comment, 4 May, p. 481), discusses the use of serum collections that are taken for one purpose and used for another. She gives the examples of the serum collections of the Harvard Hypertension and Follow-up Program that are being used to determine the relation of levels of vitamin A, vitamin E, or carotenoids to cancer. The low cost of \$6000 for the study is emphasized. She also refers to the Comstock collection of serum from 25,000 healthy persons in follow-up studies of cancer.

I was pleased to see the usefulness of serum banks recognized; however, "the new epidemiology" is neither so new nor so inexpensive as indicated. The World Health Organization has promoted the multiple uses of serum collections since 1959 (1) and has established three serum banks (2). The applications of seroepidemiology have been presented over the past 20 years, and results from the use of the Comstock collection in the seroepidemiology of Hodgkin's disease were presented in 1981 (3).

Readers should also be aware of the continuing costs of maintaining the serum collection, continuing follow-up for diseases such as cancer in prospective studies, and carrying out tests on the serum. We are currently working with the Harvard School of Public Health on a large prospective study of Hodgkin's dis-



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