Breast Cancer, Birth Cohorts, and Epstein-Barr Virus: Methodological Issues in Exploring the "Hygiene Hypothesis" in Relation to Breast Cancer, Hodgkin's Disease, and Stomach Cancer[1]

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Abstract

To address methodological issues in exploring a variant of the "hygiene hypothesis" that posits delayed infection by Epstein-Barr virus contributes to rising rates of breast cancer and Hodgkin's disease, we examined birth cohort trends in the incidence of both cancers plus stomach cancer, building on previously reported year-of-diagnosis cross-sectional associations of age-standardized rates. Using published data from the United States Connecticut state cancer registry (1935–1998) for women for each cancer site, we obtained age-specific incidence rates by birth cohort (1870–1874 to 1970–1974), along with age-standardized incidence rates for selected calendar years (1935–1939, 1940–1944, . . . , 1990–1994, 1995–1998). Clear secular trends in incidence rates, in the opposite direction, were evident for: (*a*) breast cancer and for Hodgkin's disease in young adults (increasing), and (*b*) stomach cancer (decreasing). Correlations between the incidence of breast cancer among women ages 50–54 and Hodgkin's disease among young adults (ages 20–24) were stronger for birth cohort (Pearson correlation, 0.85) than for cross-sectional analyses (Pearson correlation, 0.68). Stronger associations between the incidence of breast cancer and non-Hodgkin's disease were evident for birth cohort compared with cross-sectional analyses, findings consonant with (but not "proof" of) the hygiene hypothesis. One methodological implication is that tests of the hygiene hypothesis must take into account birth cohort effects and age at incidence of the outcomes under study; age-standardized cross-sectional analyses may be misleading.

Introduction

"Cleanliness is next to godliness," so the saying goes, or at least since the mid-1800s, it has been the sanitarians' ideal. To mitigate the unprecedented urban squalor and stench of that period, they urged provision of ample sewers and unsullied water, coupled with the promotion of hygienic living and regulation of food production (1–3); a minority also called for better wages and working conditions (1, 3). After the early 20th century's decline in acute infectious disease in both Europe and the United States and the concomitant rise in cancer and cardiovascular disease, public health attention shifted to what became termed "chronic" disease, almost always assumed to have a noninfectious etiology (2–4). This assumption, however, has been challenged by late 20th century epidemiologic research, with new studies implying not only that microbes may be implicated in the etiology of various chronic diseases but also that "cleanliness" may perhaps be taken too far. The recently dubbed "hygiene hypothesis," for example, postulates that improved sanitation, decreased crowding, and reduced family size have been enormously beneficial in reducing infant mortality and many childhood diseases, but with unanticipated consequences (5–7). In particular, rising rates of atopic asthma are posited to arise from alterations in immune system function in children no longer subject to what were once common childhood infections (5–7). One corollary, with strong methodological implications for etiologic analysis, is that there should be marked birth cohort effects for diseases the incidence of which is linked to societal changes in hygienic resources.

An opportunity to consider the impact of methodological issues in testing the "hygiene hypothesis" is provided by a recent study by Yasui *et al.* (8), who investigated whether delayed infection with EBV, one of the first identified viral carcinogens (9, 10), is a risk factor for breast cancer. Virtually universal, infection by EBV typically occurs "within a few months after birth in underdeveloped countries," but at later ages in more industrialized countries, albeit "rarely later than in the second or third decade of life in the upper socioeconomic classes" (Ref. 9, p. 573). Early childhood infection by EBV was first linked in the 1960s to Burkitt's lymphoma, after which strong associations were found between later childhood infection and both infectious mononucleosis and Hodgkin's disease among young adults (9–15). Of note, the two-part study by Yasui *et al.* observed both a 2–3-fold increased risk of breast cancer associated with first occurrence of infectious mononucleosis at age 25 or older or tonsillectomy at age 15 or older, and a strong year-of-diagnosis cross-sectional correlation between incidence of breast cancer and Hodgkin's disease among

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cancer registries both within the United States and in other countries (8).

Hypothesizing about determinants of breast cancer in relation to the hygiene hypothesis requires taking into consideration not only direct effects of changes in hygienic resources but also concomitant factors related to an increased standard of living that might affect risk of breast cancer. Also relevant is prior research and theorizing about possible viral etiologies of breast cancer, including murine mammary tumor virus (16–20). An expanded hypothesis would thus posit that the 20th century increase in breast cancer incidence reflects: (*a*) earlier age at menarche (reflecting better nutrition) and reduced childbearing (reflecting girls' increased education and women's rising employment in the paid labor force; Refs. 21, 22), and (*b*) a shift from early life to later infection by EBV (8).

Population-based research linking EBV infection and breast cancer, however, remains sparse. In the mid-1990s, a handful of cross-sectional epidemiologic studies reported EBV in malignant, but not in healthy, breast epithelial tissue in women's biopsy specimens (23–25). These findings were not confirmed by subsequent research (26–32). With regard to national comparisons, a large two-country prospective study documented associations between a prior history of infectious mononucleosis and subsequent increased risk for Hodgkin's disease but not breast cancer (33). The study by Yasui *et al.* (8) was the first to compare population-based incidence rates of breast cancer and Hodgkin's disease across multiple countries, albeit using cross-sectional data.

Nevertheless, from both an ecosocial and lifecourse perspective (34), a year-of-diagnosis cross-sectional association between the age-standardized incidence of Hodgkin's disease and breast cancer raises more questions than it answers. This is because the epidemiologic patterning of Hodgkin's disease and breast cancer by age are quite distinct. In wealthier countries, Hodgkin's disease incidence peaks among young adults (ages 25 to 30; Refs. 10, 11), whereas the incidence of breast cancer is greatest among postmenopausal women (21, 22). If both are hypothesized to be linked to a common early life exposure, then the salient referent point is year of birth, not year of diagnosis. The implication is that year-of-diagnosis cross-sectional correlations of their incidence rates could be confounded by birth cohort effects, given associations between the year of birth and the incidence rates (35). To avoid this problem, we accordingly examined trends in the incidence of breast cancer and in Hodgkin's disease among young women by birth cohort. We also included data on stomach cancer among women, because the 20th century secular decline in stomach cancer incidence in industrialized nations has been hypothesized to be caused both by improved hygiene in food manufacturing, preservation, and preparation and by reduced early childhood infection by *Helicobacter pylori* (36, 37).

Materials and Methods

Cancer Registry Data. To locate cancer registries that had collected data for a sufficiently long timespan to provide meaningful data on birth cohort trends in breast cancer incidence, we examined: (*a*) United States cancer incidence data from the Surveillance, Epidemiology and End Results (SEER) registries for all available years (1973–1998),[6] (*b*) data from the Connecticut state cancer registry, established in 1935 (38), and (*c*) data from the Cancer Incidence in Five Continents series (39–45). Because of the importance of including breast cancer incidence among women age 50 and older, we concluded that birth cohort analyses required restricting our analyses to cancer registries with data extending back prior to the calendar year 1945. We accordingly restricted our analyses to the Connecticut cancer registry data.

In accordance with the principles embodied in the Declaration of Helsinki, our study was exempted from Institutional Review Board (IRB) approval by the Harvard School of Public Health Human Subjects Committee because all of the data in our study were aggregate data derived from published sources that could not be linked back to any individuals.

Cancer Incidence Rates. The published yearly age-specific incidence rates for breast cancer, stomach cancer, and Hodgkin's disease among women from the Connecticut cancer registry were the source of our cancer incidence rates (38). For analyses of cross-sectional age-adjusted incidence rates, we employed the new United States 2000 standard million (46).

Statistical Methods. We computed 95% confidence intervals of the United States age-specific rates using two methods. When raw event counts for any age group numbered at least 30, we applied a normal approximation to the Poisson distribution (Ref. 47, pp. 681–682). In the few instances when raw event counts for any age group numbered fewer than 30, we computed exact 95% confidence intervals, using exact methods (Ref. 47, pp. 196–199). Cumulative probabilities were computed using STATA (STATA. College Station, TX: STATA 2001). Lastly, to generate data analogous to that reported by Yasui *et al.* (8), we calculated Pearson correlation coefficients for the incidence rates of the three cancers for the 12 specified calendar periods: 1935–1939, 1940–1944, . . . , 1990–1994, 1995–1998.

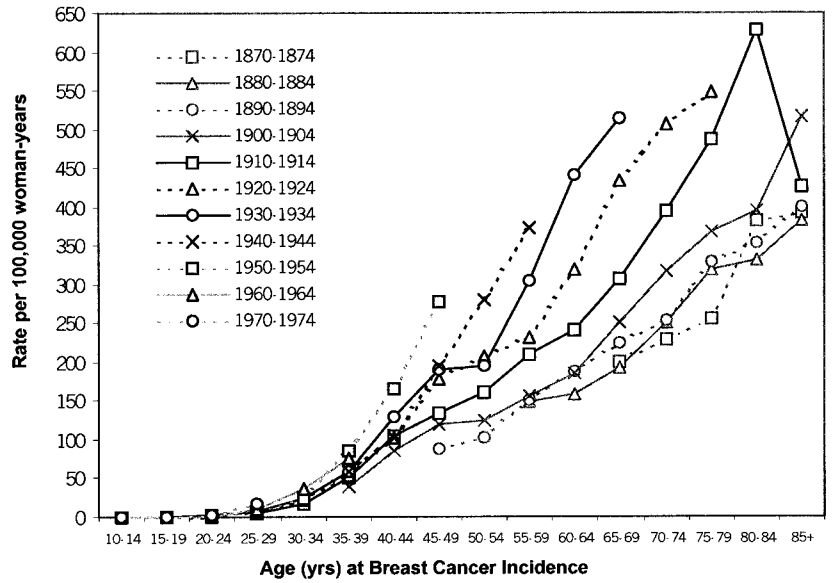
Results

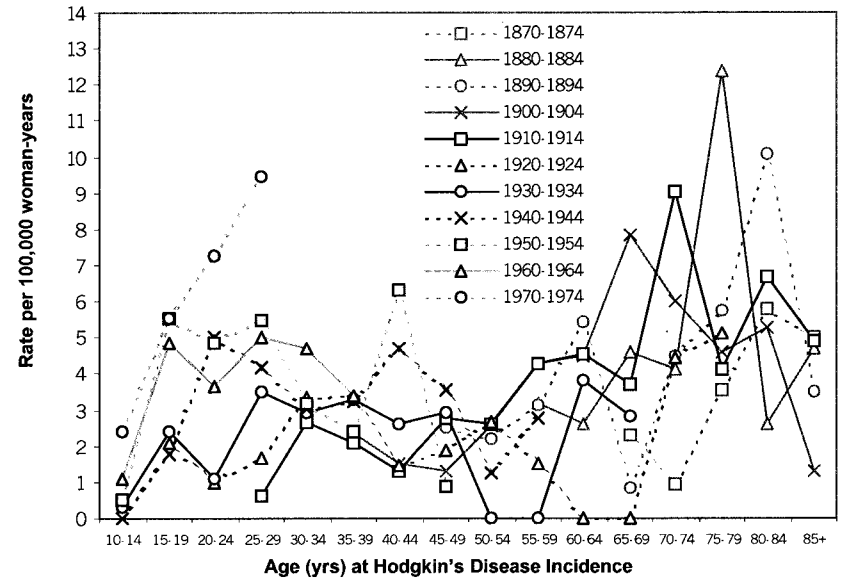
Fig. 1 presents age-specific incidence data, and Table 1 presents the rates with their 95% CIs[7] for breast cancer, Hodgkin's disease, and stomach cancer among women for the Connecticut state cancer registry. Included are all of the cases diagnosed between 1935 and 1998 and birth cohorts in selected 5-year intervals between 1870–1874 to 1970–1974. The data depict a marked increase in breast cancer incidence and a sharp decline in stomach cancer by birth cohort across the entire span of cohorts, which was especially evident across cohorts for women diagnosed at age 45 and older. For Hodgkin's disease, the data indicate a notable increase in incidence among women diagnosed between ages 15 and 29 for women in birth cohorts born in 1930 or later but reveal no clear trends in incidence by birth cohort among women diagnosed at age 45 and older.

Fig. 2 presents plots of correlations between: (*a*) the age-specific incidence of breast cancer for women ages 50–54 and for Hodgkin's among women ages 20–24 by year of birth, and (*b*) the age-standardized cross-sectional incidence rates of breast and Hodgkin's disease among women in the 12 selected calendar periods (1935–1939, 1940–1944, . . . , 1990–1994, 1995–1998). Of note, the Pearson correlation for the birth cohort analysis (r, 0.85; 95% CI, 0.26–0.98) was stronger than for the cross-sectional analysis (r, 0.68; 95% CI, 0.16–0.90), consonant with what would be expected if the hygiene hypothesis were correct.

[6] SEER Program. Public-Use Database 1973–1998, August 2000 Submission. Bethesda, MD: National Cancer Institute, 2001.

[7] The abbreviation used is: CI, confidence interval.





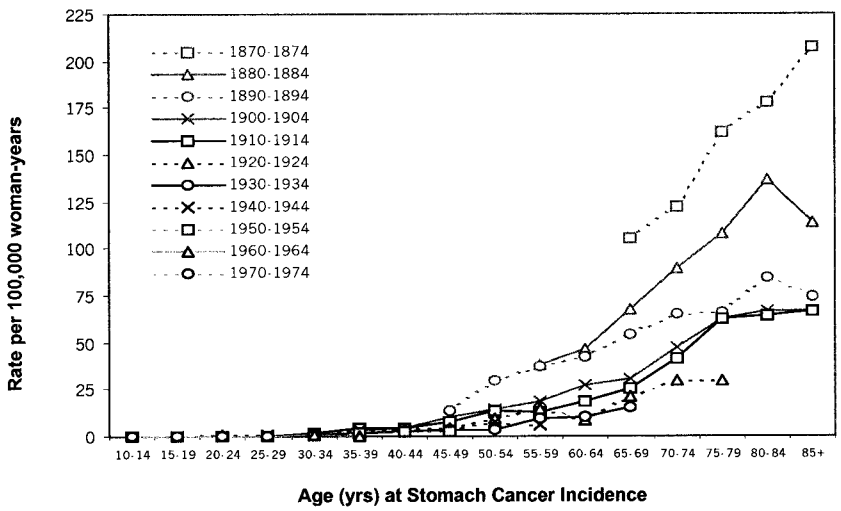


Fig. 1. Age-specific cancer incidence rates by birth cohort (1870–1874 to 1970–1974) for breast cancer, Hodgkin's disease, and stomach cancer: Connecticut, United States of America.

Table 1 Connecticut cancer incidence rates among women for breast cancer, Hodgkin's disease, and stomach cancer (cases per 100,000 women-years) and 95% CIs, among women, by age and birth cohort, 1870–1998

Cancer birth cohort	Age (years)							
	10–14	15–19	20–24	25–29	30–34	35–39	40–44	45–49
Breast cancer								
1870–1874								
1890–1894								88.8 (77.8–99.8)
1910–1914				5.6 (3.2–8.1)	17.5 (13.2–21.7)	50.4 (43.3–57.5)	103.9 (93.7–114.1)	133.3 (121.9–144.7)
1930–1934	0 (0.0–1.1)	0.3 (0.0–1.7)	2.4 (1.1–4.6)	6.7 (4.4–9.8)	24.1 (19.5–28.7)	57.3 (50.4–64.3)	128.0 (117.6–138.4)	190.2 (177.4–203.0)
1950–1954	0 (0.0–0.6)	0.3 (0.0–1.4)	1.3 (0.6–2.6)	8.0 (5.7–10.3)	24.0 (15.4–32.6)	85.7 (69.6–101.9)	165.2 (142.8–187.6)	277.4 (247.1–307.8)
1970–1974	0 (0.0–3.0)	0 (0.0–2.9)	2.4 (0.5–7.1)	17.2 (10.5–26.6)				
Hodgkin's disease								
1870–1874								
1890–1894								2.5 (1.0–5.1)
1910–1914				0.6 (0.1–2.2)	2.7 (1.3–4.9)	2.1 (0.9–4.1)	1.3 (0.4–3.0)	2.8 (1.4–5.0)
1930–1934	0.3 (0.0–1.6)	2.4 (1.0–4.8)	1.1 (0.3–2.8)	3.5 (1.9–5.9)	2.9 (1.6–5.0)	3.3 (1.9–5.5)	2.6 (1.4–4.6)	2.9 (1.6–5.0)
1950–1954	0.5 (0.1–1.5)	5.5 (3.6–7.4)	4.9 (3.1–6.6)	5.5 (3.6–7.4)	3.2 (0.9–8.2)	2.4 (0.5–7.0)	6.3 (2.7–12.5)	0.9 (0.0–4.8)
1970–1974	2.4 (0.5–7.0)	5.5 (2.2–11.4)	7.3 (3.3–13.8)	9.5 (4.7–16.9)				
Stomach cancer								
1870–1874								
1890–1894								13.1 (8.9–17.4)
1910–1914				0.3 (0.0–1.7)	1.9 (0.8–3.9)	3.9 (2.2–6.5)	4.4 (2.6–7.1)	7.6 (4.9–10.3)
1930–1934	0 (0.0–1.1)	0 (0.0–1.1)	0 (0.0–1.0)	0.2 (0.0–1.4)	1.1 (0.4–2.6)	1.5 (0.6–3.2)	2.4 (1.2–4.3)	3.1 (1.7–5.3)
1950–1954	0 (0.0–0.6)	0 (0.0–0.7)	0 (0.0–0.6)	0 (0.0–2.9)	0.0 (0.0–2.9)	1.6 (0.2–5.7)	2.4 (0.5–6.9)	2.6 (0.5–7.6)
1970–1974	0 (0.0–3.0)	0 (0.0–2.9)	0 (0.0–3.0)	0 (0.0–3.2)				

Cancer birth cohort	Age (years)							
	50–54	55–59	60–64	65–69	70–74	75–79	80–84	85+
Breast cancer								
1870–1874				199.2 (175.1–223.3)	228.9 (200.5–257.3)	255.2 (221.1–289.4)	383.0 (329.6–436.3)	392.9 (331.5–454.4)
1890–1894	102.2 (90.3–114.2)	151.0 (136.0–166.1)	186.6 (169.3–203.9)	225.0 (205.7–244.3)	253.9 (231.8–275.9)	329.1 (300.7–357.1)	353.2 (319.5–387.0)	398.5 (361.6–435.3)
1910–1914	161.6 (148.8–174.4)	208.5 (193.8–223.1)	241.1 (224.8–257.3)	307.1 (288.0–326.3)	393.9 (341.6–446.1)	487.9 (425.5–550.3)	627.8 (546.8–708.8)	425.7 (362.3–489.1)
1930–1934	194.7 (166.1–223.3)	303.6 (266.9–340.2)	441.3 (394.7–487.8)	514.9 (462.0–567.8)				
1950–1954								
1970–1974								
Hodgkin's disease								
1870–1874				2.3 (0.5–6.7)	0.9 (0.0–5.1)	3.6 (0.7–10.4)	5.8 (1.2–17.0)	5.0 (0.6–18.1)
1890–1894	2.2 (0.8–4.8)	3.1 (1.4–6.2)	5.4 (2.9–9.3)	0.9 (0.1–3.1)	4.5 (2.1–8.5)	5.7 (2.6–10.9)	10.1 (5.2–17.6)	3.5 (1.0–9.1)
1910–1914	2.6 (1.3–4.8)	4.3 (2.4–6.9)	4.6 (2.6–7.4)	3.7 (1.9–6.5)	9.0 (2.9–21.1)	4.2 (0.5–15.0)	10.9 (3.0–27.8)	4.9 (0.6–17.8)
1930–1934	0.0 (0.0–4.0)	0.0 (0.0–4.2)	3.8 (0.8–11.2)	2.8 (0.3–10.2)				
1950–1954								
1970–1974								
Stomach cancer								
1870–1874				105.7 (88.1–123.3)	122.3 (101.5–143.0)	161.5 (134.3–188.6)	177.9 (141.6–214.3)	207.7 (163.0–252.4)
1890–1894	29.2 (22.8–35.6)	37.2 (29.7–44.6)	42.5 (34.2–50.7)	53.7 (44.3–63.1)	65.0 (53.8–76.1)	65.6 (52.9–78.2)	83.9 (67.5–100.3)	74.5 (58.6–90.5)
1910–1914	13.2 (9.5–16.8)	12.8 (9.2–16.4)	18.5 (14.0–23.0)	25.4 (19.9–30.9)	41.6 (26.3–62.4)	62.3 (40.0–84.6)	43.5 (24.9–70.6)	66.4 (41.4–91.5)
1930–1934	3.3 (0.7–9.6)	9.2 (4.0–18.1)	10.2 (4.4–20.2)	15.6 (7.8–27.8)				
1950–1954								
1970–1974								

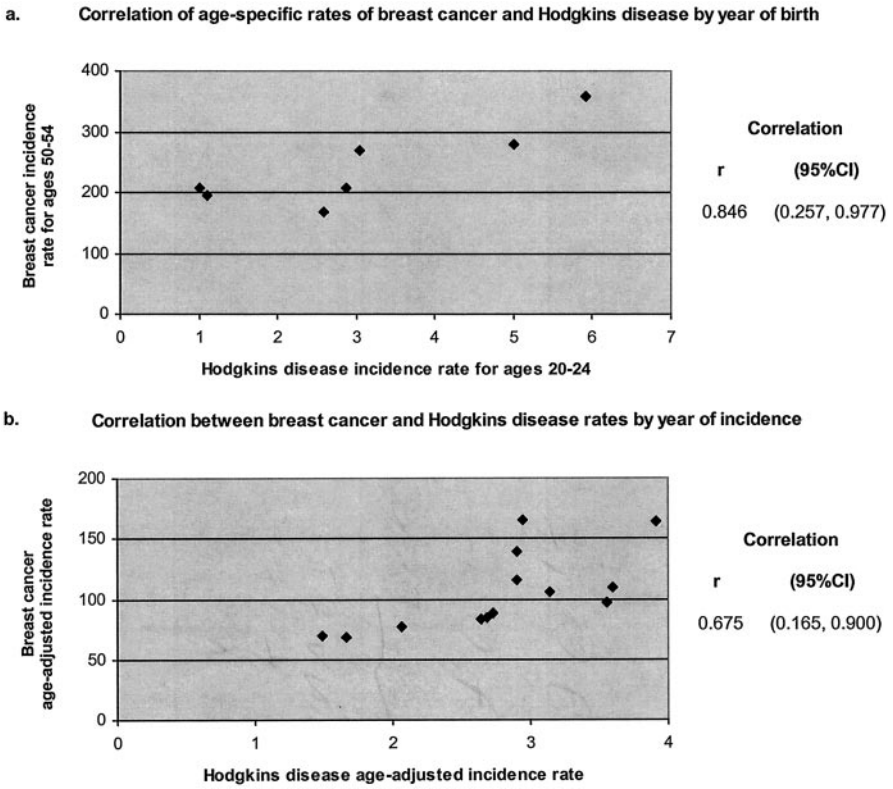


Fig. 2. Correlations among Connecticut women for (*a*) age-specific incidence rates (per 100,000 women-years) of breast cancer and Hodgkin's disease by year of birth (1915–1919 through 1945–1949), and (*b*) year-of-diagnosis age-standardized rates of breast cancer and Hodgkin's disease for 12 selected calendar years (1935–1939, 1940–1944, . . . , 1990–1994, 1995–1998).

Discussion

Our findings, based on Connecticut cancer registry data collected since 1935, provide tentative support for the hygiene hypothesis in relation to the incidence of breast cancer and Hodgkin's disease and underscore the fact that analyses correlating cancer incidence rates to test hypotheses about postulated common etiologic factors should take into account birth cohort effects and relevant age at incidence. Congruent with the hygiene hypothesis, birth cohort analyses provided stronger evidence than year-of-diagnosis cross-sectional age-adjusted analyses of associations between rising rates of breast cancer among women of all ages and of Hodgkin's disease among young women and declining rates of stomach cancer. One implication is that these two approaches to analyzing cancer incidence rates, birth cohort *versus* year-of-diagnosis cross-sectional age-adjusted analyses, can lead to different patterns of results when analyzing cancers with very different peak ages. Another is that the tests of the hygiene hypothesis pertaining to EBV infection and the risk of breast cancer will need to take into account birth cohort effects relevant to study design, data analysis, and interpretation.

Among the chief sources of error likely affecting our results are: (*a*) inaccurate registration of cancer cases, most likely leading to the underestimation of cancer incidence rates; and (*b*) inaccurate counts of the population in the catchment area, which could lead to under- or overestimation of cancer incidence rates. Although we do not possess data to ascertain the accuracy of cancer registration in Connecticut since 1935, these types of biases would likely equally affect all analyses reliant on these data and, hence, not invalidate comparisons with the registry data over time. Moreover, these biases would equally affect other analyses reliant on these data, including the cross-sectional analyses of age-standardized rates of breast cancer and Hodgkin's disease reported by Yasui *et al.* (8).

Assuming our data provide accurate depictions of birth cohort trends in the incidence of breast cancer, Hodgkin's disease, and stomach cancer, several factors could account for the observed trends. Most importantly, although the data are consistent with predictions of the hygiene hypotheses, it is critical to recognize that they are also compatible with the existence of other common etiologic factors that also vary by birth cohort. For example, the risk of breast cancer and stomach cancer vary markedly by geographic region and time period (21, 22, 48), such that secular trends of immigration (and of immigrants at what age) must be factored into any meaningful test of the hygiene hypothesis. Further complicating analyses of EBV-related associations between the incidence of breast cancer and Hodgkin's disease, recent research also suggests that EBV may be shed by breast milk (49). If true, such a pathway of transmission would be entangled with other nonhygienic pregnancy-related factors linked to a risk of breast cancer, including occurrence of pregnancy, at what age, and with what extent of breastfeeding (21, 22).

In conclusion, our findings underscore the observation that analyses of cancer incidence rates, premised on the hygiene hypothesis, should employ birth-cohort data rather than year-of-diagnosis cross-sectional age-standardized data, especially for cancers with different ages of peak incidence. The importance of considering secular trends in hygiene and household crowding in relation to birth cohort analyses of diseases with infectious etiology is additionally highlighted by marked changes in household crowding and plumbing in the United

States over the last half-century (50).[8] For example, the national proportion of crowded households (defined as having more than one person per room) dropped from 20.2% in 1940 to 4.9% in 1990.[8] Even more dramatically, the proportion of households lacking complete plumbing plummeted during this same time period from 45.3 to 1.1%. Corresponding decreases that were specific to Connecticut were from 12.9 to 2.3% for crowding, and from 18.8 to 0.4% for plumbing.[8] Future research could fruitfully assess, using birth cohort analyses, whether these trends in sanitation and household crowding are implicated in current and changing population distributions of diseases of known and hypothesized infectious etiology.

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