Epidemiologic Studies on Possible Health Effects of Intake of Pyrolyzates of Foods, with Reference to Mortality among Japanese Seventh-Day Adventists

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To elucidate the effect of intake of mutagenic and/or carcinogenic pyrolysis products of proteins and amino acids on carcinogenesis in man, we have undertaken two epidemiologic cohort studies: one concerning the possible association of broiled fish consumption with cancer and the other concerning the cancer mortality among Japanese Seventh-Day Adventists. The main findings of these studies are described.

To elucidate the effect of the mutagenic and/or carcinogenic pyrolysis products of proteins and amino acids on producing cancer in man (1), we have undertaken two epidemiologic cohort studies: one concerning the possible association of broiled fish consumption with cancer (2); and another concerning cancer mortality among the Japanese Seventh-Day Adventists, which is still in progress. The main findings of these studies will be described, although results of the second study are preliminary in nature.

Cohort Study on Association of Broiled Fish Intake and Cancer Death

Broiled fish is a very common dish in Japan and is considered to be one of the major sources of mutagenic pyrolyzates of proteins ingested by the Japanese. The Radiation Effects Research Foundation in Hiroshima examined the dietary habits of a group of adult persons in 1968, inquiring about the frequency of intake of six food items, including broiled fish, dried fish, milk, salted pickles, fruits, and rice, together with other personal characteristics, such as date of birth, sex, school career, smoking habit, and radiation dose from the atomic bomb. For a total of 7553 subjects, consisting of 2746 males and 4807 females, complete records were available for the above 11 personal characteristics (independent variables) as well as for vital status. Therefore, we followed this cohort, aged 50.1 years on the average, from January 1, 1968, up to the end of 1978. Deaths seen during the observation period were periodically checked by utilizing the so-called “ko-seki” system, confirming the vital status of all of the cohort members. Causes of death were classified by the International Classification of Diseases, 8th Revision.

The relative risk, that is, mortality, among those consuming broiled fish twice or more weekly per mortality among those consuming broiled fish less frequently, as calculated by the Mantel-Haenszel method (3), was 1.33 (p < 0.05) and 1.87 (p < 0.05) for all cancer and for stomach cancer, respectively. A similar excess mortality from liver cancer was also observed among frequent consumers of broiled fish, but with no statistical significance. Mortality rates during the observation period for cancer at all sites and cancer of the stomach were calculated by sex and age and by frequency of intake of broiled fish and examined by stepwise linear multiple regression analysis (4) for possible association with various variables, without considering interaction effects of the variables tested, using a linear model. It was revealed that intake of broiled fish makes the fourth largest contribution to deaths from cancer at all sites, being surpassed only by age, sex, and radiation dose (Table 1). It was also shown that intake of broiled fish makes the third largest contribution to deaths from stomach cancer.

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A multiple regression analysis was also made, demonstrating that intake of broiled fish is significantly and positively associated with death from cancer at all sites (Table 2). A similarly significantly positive association was also seen between the intake of broiled fish and death from stomach cancer. Other variables such as age, sex, and milk consumption were also shown to be associated with stomach cancer. Our result for milk consumption was thus contrary to the observations of Hira- yama (5) and Haenszel et al. (6) but similar to those of Yanai et al. (7), who did a multivariate analysis, as we did. At present, no reasonable explanation is available for this discrepancy. Furthermore, the relative risks for frequent intake of broiled fish calculated by the multiple regression equations were 1.3 for cancer at all sites and 1.7 for stomach cancer, both being very close to the corresponding values calculated by the Mantel-Haenszel method. These relative risks derived from the multiple regression equations cannot be explained by reference to any other independent variables tested, because these variables are controlled in the analysis. All these facts suggest that the observed slight but significant excess mortality from cancer at all sites and from cancer of the stomach as seen among frequent consumers of broiled fish is probably real.

### Table 1. Results of stepwise regression analysis of deaths from cancer at all sites and stomach cancer seen in a Radiation Effects Research Foundation cohort.*

| S. No. | Variable     | Cancer at all sites | Stomach cancer |
|--------|--------------|---------------------|----------------|
| 1      | Age          | 126.38*             | 44.85*         |
| 2      | Sex          | 80.22*              | 32.57*         |
| 3      | Radiation    | 56.87*              | 23.22*         |
| 4      | Broiled fish | 44.20*              | 18.21*         |
| 5      | Smoking      | 36.25*              | 14.82*         |
| 6      | Salted pickle| 30.70*              | 12.45*         |
| 7      | Rice         | 28.54*              | 10.71*         |
| 8      | School career| 22.30*              | 9.38*          |
| 9      | Fruit        | 20.72*              | 8.34*          |
| 10     | Milk         | 18.65*              | 7.51*          |

* Data of Ikeda et al. (2).

*p < 0.01.

### Table 2. Results of multiple regression analysis of deaths from cancer at all sites and stomach cancer seen in a Radiation Effects Research Foundation cohort.*

| Variable       | Cancer at all sites | Cancer of stomach |
|----------------|---------------------|-------------------|
| Broiled fish   | 97* (38)            | 47* (22)          |
| Milk           | 5 (23)              | 27* (13)          |
| Salted pickle  | -47 (31)            | 9 (18)            |
| Fruit          | -12 (30)            | -19 (17)         |
| Rice           | -56 (42)            | 3 (24)           |
| School career  | -23 (31)            | -5 (18)          |
| Smoking        | 73* (36)            | 4 (21)           |
| Radiation dose | 0.48* (0.16)        | 0.07* (0.09)     |
| Age at interview| 16* (2)              | 6* (1)            |
| Sex            | 161* (55)           | 92* (32)         |

*R² (%) = 2.41

### Table 3. Vital status of Seventh-Day Adventists.*

| Sex       | Alive | Dead | Unknown | Total |
|-----------|-------|------|---------|-------|
| Male      | 1944  | 95   | 315     | 2354  |
| Female    | 4506  | 197  | 636     | 5339  |

Total 6450 292 951 7693

* Number of subjects: 7693 - 951 = 6742
among this group during the observation period (but only after the fifth anniversary of conversion) was then compared with the corresponding expected number of deaths, calculated by multiplying age-sex-specific person-years at risk with age-, sex-, and cause-specific mortality rates for the Japanese population in 1975.

As shown in Table 4, the O/E ratio for all causes of death was 0.77 for males and 0.74 for females, which were significantly less than unity. Since, as mentioned, those whose vital status was unknown were eliminated from the present calculation, such elimination might be associated with the observed reduction in overall death. This does not seem probable, however, because: (1) the present cohort is a specific religious one, consisting of probably the most religious persons among the Japanese, and any deaths would be notified to the church without fail; (2) our failure to confirm vital status was, as mentioned, almost exclusively due to clerical oversight in recording the permanent address, and such oversight is unlikely to be associated with the health status or survival of the subjects; and (3) those subjects eliminated from the analysis because of unknown vital status and those who could be followed were comparable in sex and age composition. Thus, the overall reduced mortality observed seems real. For cancer at all sites, a highly significantly lowered mortality was observed in males, and the mortality for females was also lower than expected but to a lesser degree and without statistical significance. For cancer of the stomach, a significantly reduced mortality was observed for both males and females. For cancers of the esophagus, intestine and colon, rectum, liver, pancreas, and lung, and for leukemia, statistically significant deviations in mortality were not observed for either males or females. Mortality from cancer of the respiratory system was found to be significantly reduced only for males.

For diseases other than cancer, a notably reduced mortality was observed for cerebrovascular diseases in males and females. The O/E ratio for ischemic heart disease was 1.0 in males but was significantly less than unity in females. No statistically significant reductions or elevations in mortality were observed for tuberculosis and diabetes in males or females.

All the above findings obtained from the cohort studies seem to suggest that mortality from cancer at all sites and from cancer of the stomach might be associated with the intake of pyrolysis products of proteins and amino acids. However, the evidence is still weak and vague, and no conclusive remarks can be made. Further investigations may provide further support for our findings.

The question as to whether or not the pyrolysis products of foods are carcinogenic to man will be very hard to answer by epidemiological studies, mainly because cooking is a universal practice, and all men are exposed to mutagenic pyrolizates of foods to a substantial degree. Thus, it is probably not possible to find persons who have extremely low levels of exposure to them. Also, estimation of an individual's exposure to single mutagenic pyrolizates of food components is not yet possible. Of these two difficulties, the former seems impossible to solve in the future, because we shall continue cooking of foods. To make matters more difficult, man is exposed to complex environmental mutagens other than mutagenic food pyrolysates. It will be extremely difficult to demonstrate unequivocally by simple epidemiologic approaches any specific health effects which might be caused by intake of such pyrolizates. In this respect, Seventh-Day Adventists seem to be an extremely valuable study population, because as already mentioned, their exposure to environmental mutagens is much simpler than that of the general popu-

| Cause of death                        | Male          | Female       |
|--------------------------------------|---------------|--------------|
|                                      | Observed      | Expected     | O/E  | Observed      | Expected     | O/E  |
| All causes                           | 95            | 122.7        | 0.77*| 197           | 267.2        | 0.74*|
| All cancer                           | 7             | 23.6         | 0.30*| 36            | 46.0         | 0.78 |
| Esophagus                            | 0             | 0.9          | 0    | 0             | 1.2          | 0    |
| Stomach                              | 3             | 9.5          | 0.32*| 4             | 15.3         | 0.26*|
| Intestine and colon                  | 1             | 0.9          | 1.11 | 1             | 2.4          | 0.41 |
| Rectum                               | 0             | 1.0          | 0    | 3             | 2.2          | 1.36 |
| Liver                                | 0             | 2.1          | 0    | 3             | 3.0          | 1.00 |
| Pancreas                             | 0             | 1.0          | 0    | 3             | 1.9          | 1.54 |
| Respiratory system                   | 0             | 3.8          | 0*   | 4             | 3.9          | 1.04 |
| Lung                                 | 0             | 3.2          | 0    | 4             | 3.2          | 1.25 |
| Leukemia                             | 0             | 0.5          | 0    | 0             | 1.0          | 0    |
| Breast                               | 2             | 2.5          | 0.80 | 2             | 2.5          | 0.84 |
| All tuberculosis                     | 4             | 2.4          | 1.67 | 3             | 2.5          | 1.21 |
| Diabetes                             | 2             | 1.4          | 1.43 | 1             | 3.7          | 0.27 |
| Cerebrovascular disease              | 12            | 31.7         | 0.38*| 32            | 74.1         | 0.43*|
| Ischemic heart disease               | 18            | 18.1         | 1.00 | 29            | 43.4         | 0.67*|

*p < 0.05.

*p < 0.01.
Table 5. Comparison of Seventh-Day Adventists with general population in regard to exposure to environmental mutagens.

| Population | Tobacco | Alcohol | Coffee | Tea | Meat | Fish | Group |
|------------|---------|---------|--------|-----|------|------|-------|
| General population | + | + | + | + | + | + | A |
| SDA | - | - | + | + | + | + | B |
| - | - | - | + | + | - | + | C |
| - | - | - | - | + | + | + | D |
| - | - | - | - | - | - | - | E |
| - | - | - | - | - | - | - | F |

ulation, allowing us a more refined and precise analysis of the risk of diseases which might be associated with different dietary habits. As shown in Table 5, if we could compare the risk of diseases between group B and group F consisting of the most devoted Adventists who compose about a half of the Adventists in the United States (5,6), or between group D which consists of Adventists not strictly abstaining from meat, poultry, and fish, and group F, it might be possible to elucidate the possible effects of the intake of mutagenic pyrolyzates of foods. Unfortunately, such an epidemiologic study cannot readily be done in Japan because the number of Adventists is limited there, while it may be possible in the United States.

If the second difficulty could be overcome in the future, the epidemiologic elucidation of the present question will become far more precise and efficient. In order to clarify epidemiologically the possible health effects of the intake of a specific mutagenic compound formed by cooking, we, of course, have to know the individual exposure to such compound. This is very important in view of the fact that quite a few different mutagens have been isolated from food pyrolyzates. Good cooperation with chemical analysts seems particularly important for the epidemiologic study.

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