The effect of selenium intake on the development of pancreatic cancer was investigated in female Syrian golden hamsters. Four-week-old hamsters were divided into 2 groups according to the selenium level in their drinking water and were fed a purified diet containing less than 0.05 ppm selenium. Starting 4 weeks later, groups received 10 s.c. injections at weekly intervals of N'-nitrosobis(2-oxopropyl)amine (BOP) dissolved in saline, while controls received saline alone. When the animals were killed 18 weeks after the last injection, palpable tumors were less frequent in the high-selenium group than in animals receiving low-selenium supplement, the numbers of histologically diagnosed cancerous lesions also being significantly reduced by high selenium intake. The selenium level and glutathione peroxidase activity in serum and pancreas were significantly greater in the high-selenium group. Moreover, selenium levels and glutathione peroxidase activity were both significantly higher in tumor-bearing tissue. The results suggest that glutathione peroxidase is involved as an intermediate factor in prevention of carcinogenesis by selenium.

Selenium is an essential trace element for animals. Although high doses are toxic, deficiency causes muscular dystrophy and liver necrosis. Low selenium concentration in the serum of cancer patients has been reported by Broghamer et al. (1976), along with an inverse relationship between serum selenium level and cancer mortality rates (Shamberger et al., 1976). Low serum selenium levels are also associated with an increased risk of gastrointestinal tumor development (Jacob et al., 1981; Pence and Buddingh, 1985; Reddy et al., 1985) and other cancers (Baldwin and Palkner, 1987; El-Bayoumy, 1985; Goodwin et al., 1985) have been observed in animal experiments. Spontaneously developing cancers have also been experimentally induced (Strausser et al., 1976). Selenium thus appears to play a role in development of some types of cancer.

In the present study, we examined the effects of selenium supplementation on induction of pancreatic cancers in Syrian golden hamsters by BOP. In this model, the cancers which originate from the pancreatic duct are histologically and biologically very similar to those in humans (Pour, 1985; Pour et al., 1974, 1975).

MATERIAL AND METHODS

Animals

Weaned female Syrian golden hamsters, 4 weeks of age, from Kitayama Labs (Kyoto, Japan) were randomly assigned to experimental groups. They were housed in polycarbonate cages (3 hamsters/cage) on white flake bedding (Japan Charles River, Tokyo) under constant conditions (12hr light and 12hr dark; 22 ± 1°C; 55 ± 5% relative humidity). The white flake bedding contained less than 0.02 ppm selenium.

Diets

A purified casein-based diet (Table I) was prepared (Oriental Yeast, Tokyo) and stored at 4°C for no longer than 2 months before use. The selenium level in this diet was below 0.05 ppm.

Drinking water

Drinking water was prepared every 2 or 3 days by adding appropriate amounts of sodium selenite (Nakarai, Kyoto, Japan) to distilled water to give 0.1 ppm and 4.0 ppm of selenium.

Experiment 1

One hundred and four hamsters were randomly divided into 2 groups and given water with either a low (0.1 ppm), or a high selenium content (4.0 ppm). All hamsters were fed purified diet replaced every 2 or 3 days.

Starting at 8 weeks of age, 42 hamsters from each group received 10 s.c. injections, at weekly intervals, of 10 mg/kg body weight of BOP (Nakarai) dissolved in 0.9% NaCl solution. The remaining 10 hamsters from each group were similarly given saline alone as controls.

Hamsters were examined and weighed every week. Approximate water consumption values were calculated from the decrease in water bottles. Eighteen weeks after the last injection, all hamsters were killed under ether anesthesia. The pancreas was removed, weighed, flattened after careful macroscopic examination, fixed in 10% buffered formalin, cut into 3 parts (gastric, splenic, duodenal lobes), and embedded in paraffin. Three sections from each part were stained with hematoxylin and eosin for histological examination.

Tumor incidences were analyzed using the χ² test and the numbers of tumors developing per hamster were compared using Student's t test.

Experiment 2

Fifty-five 4-week-old female hamsters were divided into 2 groups based on drinking water selenium levels. Four weeks later, hamsters in each group were further divided into BOP-treated and saline controls as in Experiment 1. Five hamsters from each group were killed at the start and at weeks 4, 13 and 31. Blood was collected by cardiac puncture and pancreas tissue was surgically removed. Both were used for assays of selenium and glutathione peroxidase levels. Pancreas tissues from both high- and low-selenium BOP-treated groups taken at week 31 were further divided into 2 categories, one with and another without tumor involvement. They were assayed separately for selenium and glutathione peroxidase. Selenium concentration was determined fluorometrically (Watkinson, 1966) as follows; serum or homogenized pancreatic tissues were digested by nitric, perchloric and sulfuric acid, heated to 100°C with 2,3-diaminonaphthalene (Aldrich, Milwaukee, WI) and extracted with cyclohexane. Fluorescence was measured at 365 nm excitation and 520 nm emission using a spectrophotometer.
(Hitachi 650-10M; Tokyo). Glutathione peroxidase (both selenium-dependent and independent) was assayed by a modification of the method of Paglia and Valentine (1967) with terbutyl hydroperoxide (Nakarai) as peroxidase substrate, coupled to NADPH (Kohjin, Tokyo, Japan) oxidation via glutathione reductase (Kohjin), and expressed as mol NADPH oxidized per g protein. Protein was measured by the method of Lowry et al. (1951). Statistical analysis was carried out with Student's $t$ test.

RESULTS

Body weights

Hamsters given low selenium supplementation were heavier than those given water with a high selenium content until the 26th experimental week, whereafter no differences were apparent (Fig. 1).

Selenium intake

Daily selenium intake per hamster calculated from water consumption was significantly higher in the high-selenium groups (average of 43.5 $\mu$g) than in the low-selenium groups (1.4 $\mu$g), although water consumption was consistently higher in the low-selenium groups.

Tumor incidence

The hamster pancreas is quite thin, relatively large tumors being fairly easily palpable. Tumors which were verified histologically were called palpable cancers. Tumors detected histologically that were too small for detection by palpation were designated histological tumors, and classified into benign and malignant categories. One hamster in the low-selenium group died very early during the experiment. Pancreatic tissues in all BOP-treated hamsters showed fatty degeneration. No such lesions or tumors were found in any of the saline-treated animals, in either the low- or high-selenium groups. Development of palpable tumor was inhibited in the high-selenium groups (Table II). The incidence of palpable cancers was also low in the high-selenium group but the incidence of histological cancer was almost the same.

The number of palpable tumors per hamster and the number of palpable tumors per palpable-tumor-bearing hamster were lower in the high-selenium than in the low-selenium groups.

The numbers of histologically diagnosed tumors per hamster, and the numbers of malignant tumors per hamster and per malignant-tumor-bearing hamster were significantly lower in the high-selenium experimental group (Table III).

The numbers of ductular adenocarcinomas, the type predominating as human pancreatic cancers, were per hamster and per malignant tumor bearing hamster significantly lower in the high selenium group. There was one mucin-producing adenocarcinoma, bearing better prognosis in the human case in the high selenium fed hamsters. Two acinar adenocarcinomas were found in the low selenium group, but none in the high selenium group.

Serum selenium levels and glutathione peroxidase activity

The serum selenium levels were significantly higher in the high-selenium group (Fig. 2). Comparison of selenium levels between saline-treated and BOP-treated animals revealed relatively lower selenium levels in BOP-treated animals except in the high-selenium group at week 13 and the low-selenium group at week 31, where BOP-treated animals showed higher selenium levels. At week 4, when the administration of BOP was initiated, significant differences in serum selenium levels were already seen between the low- and high-selenium groups. Serum glutathione peroxidase activity was also higher in the high-selenium than in the low-selenium group. There were significant differences in both values in the low- and high-selenium groups at week 4, the saline-treated low- and high-selenium groups at week 31, and the BOP-treated low- and

**TABLE I - DIET COMPOSITION**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (Vitamin-free)</td>
<td>18.0</td>
</tr>
<tr>
<td>Dextrin</td>
<td>49.5</td>
</tr>
<tr>
<td>Glucose</td>
<td>17.0</td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>6.0</td>
</tr>
<tr>
<td>Corn oil</td>
<td>3.34</td>
</tr>
<tr>
<td>Lard</td>
<td>2.66</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mixture</td>
<td>1.0</td>
</tr>
<tr>
<td>FeSO₄.7H₂O</td>
<td>0.028</td>
</tr>
</tbody>
</table>

1Mineral mixture composed of: (g/kg) CaCO₃, 207; CaHPO₄, 323; MgSO₄, 66; KCl, 209; Na₂HPO₄, 186; CuSO₄, 0.37; ferric citrate, 4.31; MnSO₄, 4-H₂O, 0.03; K₂SO₄, 0.029; ZnCO₃, 0.64L; vitamin mix in the final diet: (mg/kg) p-aminobenzonitrile, 110; ascorbic acid, 991; biotin, 0.44; vitamin B₁₂, 0.0297; calcium panthothenate, 66; choline chloride, 1920; folic acid, 1.98; inositol, 110; menadione, 49.6; niacin, 90; pyridoxine, 22; riboflavin, 22; thiamine, 22; (units/kg) vitamin A acetate, 19,824; vitamin D₃, 2,202; vitamin E acetate, 121.

**Figure 1** – Body weight of hamsters treated with BOP given 0.1 ppm and 4.0 ppm selenium supplements in drinking water.
high-selenium groups at week 31 (Fig. 3). Serum glutathione peroxidase activity increased until the 4th experimental week, and thereafter decreased in all groups.

Selenium levels and glutathione peroxidase activity in the pancreas

Selenium levels in pancreatic tissues were significantly higher in the high-selenium groups than in the low-selenium groups, and the BOP-treated animals also demonstrated lower selenium levels than the saline-treated animals (Fig. 4). Selenium levels in the pancreas were highest at the beginning of the experiment, and decreased by 4 weeks, then remaining at almost the same levels until the end of the experiment. Glutathione peroxidase activity in the pancreas was also significantly higher in high-selenium groups than in low-selenium groups, and BOP-treated groups showed less activity than the controls, the same pattern as for selenium levels being apparent. However, the activity increased until week 13 in the high-selenium group, and until week 4 in the low-selenium group (Fig. 5). At week 13, the 0.9% NaCl-treated high-selenium group had significantly greater activity than the BOP-treated high-selenium group.

Selenium levels and glutathione peroxidase activity in tumor- and non-tumor-bearing pancreatic tissue

The selenium levels of tumor-bearing tissue were significantly higher than those of non-tumor sites in both the high- and low-selenium groups (Fig. 6). Selenium levels were significantly higher in the high-selenium group than in the low-selenium group in both tumor sites and non-tumor sites. Glutathione peroxidase activity was significantly higher in tumor sites than in non-tumor sites (Fig. 7). The high-selenium group had higher glutathione peroxidase activity than the low-selenium group in both tumor-bearing and non-tumor-bearing sites, the latter demonstrating a significant difference. Both selenium level and glutathione peroxidase activity in normal control pancreas showed values intermediate between those of tumor-bearing and non-tumor-bearing sites.

**FIGURE 2** - Serum selenium levels in hamsters given high selenium supplementation and treated with (a) or without BOP (b) and in hamsters given low selenium supplementation with (c) or without BOP (d). Significant differences between the high and low selenium groups at: a, p < 0.01; b, p < 0.001. *, statistical difference from BOP treatment value (p < 0.001).

**DISCUSSION**

Many researchers have reported an inverse relationship between cancer death rate and blood selenium levels (Clark, 1985; Kogata et al., 1988). In areas where the cancer death rate is high, inhabitants have low blood selenium levels (Shamberger and Willis, 1971; Shamberger et al., 1976). Furthermore, a negative correlation with cancer stage has been observed (Kogata et al., 1988), i.e., the more advanced the tumor, the lower the selenium level of the patient. Whether the low blood selenium level in cancer patients is the cause or the result of cancer, however, remains controversial (Robinson et al., 1979). Prospective cohort studies of serum selenium after several years of frozen storage show that low-level individuals have a higher risk of upper gastro-intestinal cancer (Knekt et al., 1988; Salonen et al., 1985; Willett et al., 1983). Animal experiments aimed at assessing protective effects against skin, colon, mammary and gastric cancer suggest that selenium may indeed prevent such cancers. In our study, selenium was found to exert an inhibitory influence on induction of pancreatic can-

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**TABLE II - INCIDENCE AND FREQUENCY OF BOP-INDUCED PANCREATIC TUMORS IN HAMSTERS RECEIVING 0.1PPM AND 4.0PPM SELENIUM**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effective number</th>
<th>Palpable tumors</th>
<th>Palpable cancers</th>
<th>Histological cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ppm</td>
<td>41</td>
<td>34 (83)</td>
<td>1.88 ± 1.47</td>
<td>2.26 ± 1.31</td>
</tr>
<tr>
<td>4.0 ppm</td>
<td>42</td>
<td>29 (69)</td>
<td>1.43 ± 1.28</td>
<td>2.07 ± 1.01</td>
</tr>
</tbody>
</table>

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**TABLE III - HISTOPATHOLOGICAL DATA FOR TUMORS FROM HAMSTERS SUPPLEMENTED WITH 0.1PPM AND 4.0PPM SELENIUM IN THE DRINKING WATER**

<table>
<thead>
<tr>
<th>Selenium concentration</th>
<th>Total histologically diagnosed tumors</th>
<th>Tumor number/effective hamster</th>
<th>Benign tumors</th>
<th>Adenomas</th>
<th>Malignant tumors</th>
<th>Malignant tumors/effective hamster</th>
<th>Ductular adenocarcinomas</th>
<th>Ductular adenocarcinomas/effective hamster</th>
<th>Ductular adenocarcinomas/malignant-tumor-bearing hamster</th>
<th>Mucinous adenocarcinomas</th>
<th>Acinous adenocarcinomas</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 ppm</td>
<td>142</td>
<td>3.46 2.21</td>
<td>0</td>
<td>0</td>
<td>142</td>
<td>3.46 2.19</td>
<td>140</td>
<td>3.41 2.17</td>
<td>3.89 2.46</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>4.0 ppm</td>
<td>93</td>
<td>&lt;0.02</td>
<td>&lt;0.02</td>
<td>&lt;0.01</td>
<td>1.47 2.69</td>
<td>&lt;0.02</td>
<td>1.28 1.86</td>
<td>&lt;0.01</td>
<td>0.12 0.18</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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1. Percentage incidence values in parentheses. 2. Number per hamster. 3. Palpable tumors per palpable-tumor-bearing animal. 4. Palpable cancers per palpable-cancer-bearing animal.
FIGURE 3 - Serum glutathione peroxidase activity in hamsters given high selenium supplementation and treated with (●) or without BOP (○) and in hamsters given low selenium supplementation and treated with (■) or without BOP (□). Significant differences between the high- and low-selenium groups at: a, $p < 0.05$; b, $p < 0.02$; c, $p < 0.01$.

FIGURE 4 - Pancreatic selenium levels in hamsters given high selenium supplementation and treated with (●) or without BOP (○) and in hamsters fed low-selenium water treated with (■) or without BOP (□). Significant differences between the high- and low-selenium groups at a, $p < 0.001$.

FIGURE 5 - Pancreatic glutathione peroxidase activity in hamsters given high selenium supplementation and treated with (●) or without BOP (○) and in hamsters given low selenium water treated with (■) or without BOP (□). Significant differences between the high- and low-selenium groups at: a, $p < 0.02$; b, $p < 0.001$. *, statistical difference from BOP treatment ($p < 0.05$).

FIGURE 6 - Selenium levels in tumor- and non-tumor-bearing pancreatic tissues from BOP-treated hamsters and normal pancreas from saline-treated hamsters. Significant differences at: a, $p < 0.01$; b, $p < 0.001$; NS, not significant.
Selenium protection against pancreatic cancer

This is in contrast to the results of Birt et al. (1986, 1988) who did not observe inhibition of pancreatic cancer by selenium; however their incidences were low because of low BOP dosage. Of particular importance in the present results was the finding that the development of ductular adenocarcinomas, similar in biological character and histopathology to the predominant pancreatic tumor type in man, was significantly reduced. Thus, a clear inhibitory effect was observed although it cannot and/or "promotion" stages of carcinogenesis (Baldwin and Parker, 1987, Ip, 1981; Ip and Daniel, 1985; Thompson et al., 1984).

Many factors may be involved in the mechanisms underlying protection against cancer. The enzyme glutathione peroxidase, of which selenium is an essential constituent, exerts anti-oxidative properties and can metabolize hydroperoxides and prevent the generation of OH-. It thereby protects cells from oxygen radicals which are considered capable of playing positive roles in carcinogenesis. While glutathione is also essential for an increase in this enzyme level to occur, and other factors such as cadmium levels may interfere with its synthesis, the fact that glutathione peroxidase activity was elevated along with selenium in the high-selenium group of the present experiment does suggest a direct relationship between the two.

As expected, serum selenium levels in the high-selenium group were much higher than those in animals receiving only low selenium supplementation. The significantly higher selenium levels in the high-selenium saline-treated hamsters compared to the respective BOP-treated animals at week 31, in view of the results for tumor-bearing tissue, may suggest the sequestration of selenium by cancer cells.

Accumulation of selenium by tumors has also been reported by others (Kogata et al., 1988) who, as in the present case, measured selenium levels in both cancerous tissue and tissue distant from the cancers. This may result in a decrease of selenium in the rest of the body, e.g. in the blood, although there is disagreement on this point (Shamberger et al., 1973). A possible explanation of high selenium levels in tumors might be a tendency for immature tissues to accumulate selenium: in this respect the observation that the selenium levels in juvenile pancreas at the beginning of the experiment were high is of interest. Like selenium levels, glutathione peroxidase activity was highest in cancer-bearing tissue. Some tumors have been reported to have increased glutathione peroxidase activity, implying decreased susceptibility to anti-cancer drugs which generate peroxides (Carmichael et al., 1988; Lewis et al., 1988). Indeed, glutathione peroxidase is thought to serve as a natural protective agent against oxidative degradation in tumor cells (Thomas and Girotti, 1989), allowing them to resist free radicals generated by leukocytes and macrophages which are thought to protect the host from neoplasms. Whether the observed alteration in the ratios for selenium/glutathione peroxidase between tumor- and non-tumor-bearing tissue in the present case could have exerted some post-initiation influence remains unclear.

Other possibilities concern inhibition of enzymatic conversion of carcinogen to ultimate forms (Harbach and Swenberg, 1981), enhancement of immunological response (Spallholz et al., 1975), altered DNA repair (Lawson and Birt, 1983), and increase in the duration of all cell stages, mainly at G2 (LeBoeuf and Hoekstra, 1985; Medina et al., 1983b), as well as inhibition of protein (Vernie et al., 1979) and DNA synthesis (Medina and Oborn, 1984).

Further investigations are clearly required to elucidate the relation of selenium to cancer. With regard to clinical use, there are difficulties in practical application, because of the very narrow safe dose range, although it can also be used for reducing the toxicity of chemotherapy (Sundström et al., 1989) and possibly for the nutritional assessment of patients (Pothier et al., 1987).

ACKNOWLEDGEMENTS

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