Preventive Effect of Soybean Resistant Proteins against Experimental Tumorigenesis in Rat Colon

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(Received April 4, 1999)

Summary The insoluble 'high-molecular-weight' fraction (HMF) centrifugally separable after digestion of soy protein isolate with a microbial protease of the exo-type, of which about a quarter is regarded as an indigestible 'resistant protein,' was examined for its preventive effect against colonic tumorigenesis in a model system with male F-344 rats. The rats were intraperitoneally injected with azoxymethane (15 mg/kg BW) once a week for 3 wk and were fed a 20.6% HMF diet (+0.4% DL-Met) or 14.7% casein diet (+0.3% DL-Met) supplemented with 0.2% sodium deoxycholate (DCA) or without supplementation. Twelve wk later, 5 rats of each group were inspected for formation of tumors but no tumors were visible to the naked eye. The DCA-fed casein group was conspicuous for a low count of aberrant crypt foci. At 39 wk, 6 rats of the DCA-fed casein group (n=10) and 3 rats of the DCA-fed HMF group (n=9) had a total of 18 tumors with a major axis of 4.0±0.4 mm and 3 tumors with an axis of 2.0±0.1 mm, respectively, in contrast to only a single tumor for the DCA-unfed casein group (nil for the DCA-unfed HMF group). The difference in tumor number and size was considered significant between these DCA-fed casein and HMF groups; that is to say, HMF feeding retarded tumor development despite the frequent occurrence of preneoplastic lesions. In addition, fecal bile acid excretion was much more elevated by HMF feeding than by casein feeding. It can be assumed from these observations that the antitumorogenicity of HMF is due to the inhibitory effect of soybean resistant proteins on reabsorption as well as the mucosal contact of bile acids in the intestine.

Key Words soybean resistant proteins, experimental tumorigenesis, rat colon, antitumorigenicity, aberrant crypt foci

An insoluble 'high-molecular-weight' fraction of Protin®-treated soy protein isolate (hereinafter, HMF) is more hypocholesterolemic than the very soy protein isolate (1-3). This rise in function is accounted for by the binding of bile acids to HMF and their fecal excretion in larger amounts. HMF is inferior in protein-nutritive value to soy protein isolate, one-fourth being locked upon as indigestible 'dietary fiber' in a broad sense (4). Such proteinous 'dietary fibers' are referred to as 'resistant proteins' which, for example, affect short-fatty acid profiles in the rat cecum (5). As for 'soybean resistant proteins,' it seems to be responsible for the bile acid-catch and -excreting capacity of HMF. In this connection, we found out that HMF feeding relative to casein feeding significantly increased the number of aberrant crypt foci (ACF) or aberrant crypts (AC) in the colon of azoxymethane-treated rats receiving dietary deoxycholate (4). It is accepted among oncologists that the enlargement of ACF or crypt multiplicity is intimately involved in later tumorigenesis (6-9). If this is the case, HMF feeding may result in multiplying colonic tumors, no matter how well hypercholesterolemia is improved thereby. The present investigation aims to verify in relation to the variation of ACF, whether colonic tumorigenesis is actually affected by long-term HMF feeding.

MATERIALS AND METHODS

Diets. Soy protein isolate (Fujipro-R from Fuji Oil Co., Osaka, Japan) was thoroughly digested at neutral pH with an exo-type protease of microbial origin (Protin FC/AC from Daiwa Kasei Co., Osaka, Japan) and centrifuged to separate an insoluble fraction, followed by freeze-drying. The dried powder thus prepared is 'HMF,' with which we were supplied in bulk by the Soy Protein Research Committee of Japan. Vitamin and mineral mixtures (AIN-76 likeness) were purchased from Oriental Yeast Co., Tokyo, Japan. Other ingredients were also commercially available. The compositions of diets are shown in Table 1.

Feeding and design. Male Fischer rats, 4 wk of age, were purchased from Japan Clea Co., Osaka, Japan, and housed in our animal-care facilities with a half-day
light/dark cycle. Fifty-five F-344 rats after 1-wk accu-
lation to the new environment were divided into 4
groups (n=10 or 15) and allowed free access to the
respective diets shown in Table 1. The bitterness of de-
oxoycholic acid was so successfully masked by coating
the sodium salt (DCA) with an enteric chemical “cellu-
lose acetate phthalate” so as not to reduce appetite.

Table 1. Composition of experimental diets (%).

<table>
<thead>
<tr>
<th></th>
<th>−DCA1</th>
<th>+DCA1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Casein</td>
<td>HMF</td>
</tr>
<tr>
<td>Casein2</td>
<td>14.7</td>
<td>20.6</td>
</tr>
<tr>
<td>HMF3</td>
<td>0.3</td>
<td>0.4</td>
</tr>
<tr>
<td>l-Methionine4</td>
<td>69.0</td>
<td>68.0</td>
</tr>
<tr>
<td>Corn starch5</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Soybean oil6</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mixture2</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin mixture2</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Cellulose powder2</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Sodium deoxycholate4</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

1 Supplemented with sodium deoxycholate (+DCA) or not
(−DCA).
2 Products of Oriental Yeast Co., Tokyo, Japan.
3 Supplied from Fuji Oil Co., Osaka, Japan; the difference in
protein source between HMF and casein diets was based
on our preliminary experiment with growing rats.
4 Chemicals of analytical grade purchased from Nacalai
Tesque, Kyoto, Japan; sodium deoxycholate was coated
on our preliminary experiment with growing rats.
5 Purchased from Kansai Denpun, Kyoto, Japan.

Laboratory Animals.

Count of preneoplastic lesions. The colons taken out
at 12 or 39 wk were immediately flushed with cold
saline, slit lengthwise, sandwiched between two sheets
of filter paper, and fixed in 10% formalin. The flattened
colons were stained with 0.2% methylene blue in Petri
dishes, followed by ACF counting under a stereoscopic
microscope at a magnification of ×30 (6).

Quantification of tumors. No tumors were observed
in any of the rat colons taken out at 12 wk, but at 39 wk,
there appeared one tumor in each of 3 rats in the
DCA-fed HMF group and plural tumors each of 6 rats
in the DCA-fed casein group. The tumors visible to
the naked eye were enumerated and their individual
sizes were measured in major-axial millimeters with a
vernier micrometer. Thus, the tumor number was not
only totaled for each group but also averaged for tumor-
bearing rats, while the tumor size was obtained as
mean±SE for all of the individual tumors in each
group.

Plasma lipid and protein assays. After the stated in-
tervals, the rats were sacrificed by blood-gathering from
the abdominal aorta under etherization. The blood
samples were immediately centrifuged in heparin-con-
taining tubes so as to separate the plasma. Plasma samples
were divided into small portions and stored at
−80°C until use. The plasma triacylglycerols, chole-
sterol and bile acid concentrations were measured using
commercially available assay kits (products of Wako
Pure Chemical Co., Osaka, Japan) by methods of enzy-
matic analysis (10–12). Total protein and albumin were
determined according to the Lowry-Folin (13) and
bromocresol-green methods (14), respectively.

Fecal lipid or steroid determination. The feces col-
clected for the last week of the 12th or 39th wk were
chunked and put in lyophilization. Part of the powdered
feces was extracted with chloroform–methanol (2:1, v/v),
and the total lipid content was estimated by weighing the oily residue after evaporation of the sol-
vent. Another part of the fecal powder was saponified in
alcoholic 1 mol/L NaOH (70°C, 60 min). After the re-
moval of neutral steroids by washing with petroleum
ether, the aqueous layer was autoclaved under strongly
alkaline conditions and then treated with chloro-
form–methyl (2:1, v/v) under acidic conditions
below pH 2 so as to extract acidic steroids. Neutral
and acidic steroids were colorimetrically determined using
assay kits for total cholesterol and bile acid measure-
ments, respectively, in the above-mentioned manner.

Statistical analysis. Data were obtained as means±
SE for the rats of each group unless otherwise noted.
Differences among groups were analysed by the
Student-Newman-Keuls test (15) following ANOVA,
and were considered significant at p<0.05. Actual cal-
culation was performed on an Apple Macintosh com-
puter using SPSS 6.1J software for Macintosh com-
puters (SPSS Japan Inc., Tokyo, Japan).

RESULTS

Table 2 summarizes the results of measurements at
Table 2. Growth parameter and plasma lipid or protein concentration in azoxymethane-treated rats at 12 wk.

<table>
<thead>
<tr>
<th></th>
<th>−DCA</th>
<th>+DCA</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Casein</td>
<td>HMF</td>
<td>Diet DCA</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>10.7±0.2</td>
<td>10.8±0.1</td>
<td>0.887</td>
</tr>
<tr>
<td>Body weight gain (g/12 wk)</td>
<td>199±5</td>
<td>198±4</td>
<td>185±5</td>
</tr>
<tr>
<td>Tissue weight (g/100 g body weight)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>3.01±0.04a</td>
<td>2.85±0.05b</td>
<td>3.38±0.09a</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.60±0.01b</td>
<td>0.60±0.01b</td>
<td>0.63±0.01a</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.23±0.01b</td>
<td>0.23±0.01b</td>
<td>0.29±0.01a</td>
</tr>
<tr>
<td>Plasma lipid concentration (mmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>1.13±0.12a</td>
<td>0.80±0.05b</td>
<td>0.45±0.02c</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.48±0.05b</td>
<td>0.96±0.04c</td>
<td>1.97±0.15a</td>
</tr>
<tr>
<td>Plasma protein concentration (g/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>71.0±0.99</td>
<td>59.2±0.5</td>
<td>65.1±1.2b</td>
</tr>
<tr>
<td>Albumin</td>
<td>39.2±0.4a</td>
<td>36.7±0.3b</td>
<td>41.1±0.5a</td>
</tr>
</tbody>
</table>

Male Fischer rats weighing about 50 g were treated with azoxymethane and divided into 4 groups, which were allowed free access to their respective diets. At 12 wk, five rats were arbitrarily picked from each group and blood samples were collected from the abdominal aorta under etherization, followed by plasma biochemical assays in the usual way. Values are means±SE (n=5); those not sharing a common superscript in the same row are significantly different at p<0.05.

12 wk regarding daily food intake, body weight gain, tissue weight, and plasma lipid and protein concentrations. There were no significant differences among the 4 groups regarding food intake and body weight gain, while the plasma cholesterol concentration was significantly lowered by HMF intake relative to casein intake in both cases with and without DCA supplementation, which was in accordance with our expectations. The same was observed for plasma triacylglycerol concentration in HMF groups as compared with the DCA-unfed casein group. Supplementation of the casein diet with DCA led to a 50% reduction in plasma triacylglycerol concentration, being in odd contrast to the somewhat swollen liver of this group. There was a significant difference in total protein concentration between DCA-unfed casein and HMF groups but the difference was abolished by supplementation of the diets with DCA. The plasma albumin concentration in the casein group was not significantly different from that in the HMF group in either case, without or with DCA supplementation.

Figure 1 illustrates the amounts of total lipid, neutral steroid and acidic steroid excretion into the feces during the last week of the 12-wk period. The total lipid level was significantly higher in the HMF groups than in the casein groups, but fecal excretion was not affected by supplementation with DCA. Supplementation of HMF or casein diet with DCA elevated fecal acidic steroid excretion to a greater or lesser degree, the difference between both dietary groups also being significant irrespective of DCA supplementation. Although there was a significant difference in fecal neutral steroid excretion between the HMF and casein groups, the excretory level in the HMF group was not elevated any further by supplementation with DCA.

The results of inspection for preneoplastic or carcinomatous (if any) lesions on the colonic mucosae at 12 wk are tabulated in Table 3. At this point in the feeding period, tumor appearance was not yet recognizable. In the meantime, numerous ACF were ranked in five cate-
Table 3. Inspection for preneoplastic lesions at 12 wk after the first azoxymethane injection.

<table>
<thead>
<tr>
<th></th>
<th>−DCA Casein</th>
<th>−DCA HMF</th>
<th>+DCA Casein</th>
<th>+DCA HMF</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aberrant crypt foci (ACF_{m,n})*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACF_{1,2}</td>
<td>10.4±2.0a</td>
<td>11.0±1.7a</td>
<td>1.2±0.7b</td>
<td>8.8±2.7ab</td>
<td>0.048</td>
</tr>
<tr>
<td>ACF_{2,4}</td>
<td>11.6±2.1b</td>
<td>10.6±2.1b</td>
<td>1.8±1.1b</td>
<td>17.6±4.3a</td>
<td>0.010</td>
</tr>
<tr>
<td>ACF_{3,8}</td>
<td>5.6±1.6b</td>
<td>5.0±1.5b</td>
<td>&lt;0.1b</td>
<td>11.2±2.8a</td>
<td>0.009</td>
</tr>
<tr>
<td>ACF_{6,16}</td>
<td>1.6±0.9b</td>
<td>3.4±0.7b</td>
<td>&lt;0.1b</td>
<td>3.6±0.9a</td>
<td>0.003</td>
</tr>
<tr>
<td>ACF_{9,32}</td>
<td>0.1±1b</td>
<td>3.2±1.2b</td>
<td>&lt;0.1b</td>
<td>2.4±0.8b</td>
<td>0.001</td>
</tr>
<tr>
<td>Total</td>
<td>29.2±1.2a</td>
<td>33.4±1.4b</td>
<td>0.0±0.4b</td>
<td>43.6±2.3a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aberrant crypts</td>
<td>100±20b</td>
<td>143±18b</td>
<td>8.8±5.6c</td>
<td>187±36a</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* ACF_{m,n}: the number of foci with (m+n) crypts per focus for each group. Values are means±SE (n=5); those not sharing a common superscript in the same row are significantly different at p<0.05.

Table 4. Growth parameter, fecal lipid and steroid excretion, and plasma lipid and steroid concentration in azoxymethane-treated rats at 39 wk.

<table>
<thead>
<tr>
<th></th>
<th>−DCA Casein</th>
<th>−DCA HMF</th>
<th>+DCA Casein</th>
<th>+DCA HMF</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food intake (g/d)</td>
<td>11.9±0.4</td>
<td>10.8±0.5</td>
<td>12.1±0.3</td>
<td>11.3±0.6</td>
<td>0.013</td>
</tr>
<tr>
<td>Body weight gain (g through 39 wk)</td>
<td>300±9a</td>
<td>283±8ab</td>
<td>268±4b</td>
<td>284±8ab</td>
<td>0.985</td>
</tr>
<tr>
<td>Fecal excretion (mg/d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total lipids</td>
<td>39±11b</td>
<td>156±2a</td>
<td>53±17b</td>
<td>175±7a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutral steroids</td>
<td>3.5±0.9b</td>
<td>6.2±0.2b</td>
<td>7.2±0.5a</td>
<td>5.1±0.1bc</td>
<td>0.557</td>
</tr>
<tr>
<td>Acidic steroids</td>
<td>6.4±2.1a</td>
<td>16.2±3.4b</td>
<td>10.3±0.2a</td>
<td>27.7±1.9b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol (mmol/L)</td>
<td>0.95±0.09a</td>
<td>0.82±0.10ab</td>
<td>0.45±0.06b</td>
<td>0.92±0.12a</td>
<td>0.090</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>2.69±0.12a</td>
<td>1.26±0.04c</td>
<td>1.73±0.10b</td>
<td>1.38±0.06bc</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bile acid (μmol/L)</td>
<td>34.0±2.6b</td>
<td>30.8±3.1b</td>
<td>62.9±5.6b</td>
<td>27.3±2.5b</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The survival rats at 39 wk were subjected to blood-gathering under etherization and their individual feces were collected for a week before the final disposition. Values are means±SE (n=5–19); those not sharing a common superscript in the same row are significantly different at p<0.05.

Categories in order of crypt multiplicity (crypts/focus). The count of total and individual ACF brought to light a strange fact that a sharp reduction by dietary DCA did not occur in the HMF groups but did occur in the casein groups. Such unexpectedly low counts of ACF and AC characteristic of the DCA-fed casein group were again ascertained in all cases at 4, 8 and 12 wk by our own reproductive experiment (data not shown).

Table 4 summarizes the results of measurements at 39 wk regarding daily food intake, body weight gain, fecal lipid and neutral steroid and acidic steroid excretion, and plasma triacylglycerol and cholesterol and bile acid concentration. There was no significant difference in food intake among all of the groups. In respect of body weight gain, a significant difference was observed between the DCA-fed and -unfed casein groups, but not between the two HMF groups nor between the HMF and casein groups, irrespective of the presence or absence of dietary DCA. Plasma cholesterol concentration was significantly lowered by HMF intake relative to casein intake in the DCA-unfed groups, but no significant difference was observed for plasma cholesterol concentration in the DCA-fed groups. On the other hand, the plasma bile acid concentration was highest in the DCA-fed casein group, being significantly different from that in other groups; the reverse was observed for plasma triacylglycerol concentration. Fecal lipid and steroid excretions were higher in the HMF groups than in the casein groups.

The results of inspection for AC, ACF and protuberant tumors on the colonic mucosae at 39 wk are tabulated in Table 5. ACF and AC both increased in number at 39 wk relative to 12 wk but the increase was not as much in ACF as in AC. Although similar increments were observed for ACF and AC in the DCA-fed casein groups, their numbers at 39 wk ranged from 8 to 15% of those in other groups. Nevertheless, 6 of 10 rats fed the DCA-supplemented casein diet possessed a total of 18 tumors with a major axis of 4.7±0.4 mm, while 3 of 9 rats fed the DCA-supplemented HMF diet possessed a total of 3 tumors with a major axis of 2.0±0.1 mm. With regard to the DCA-unfed groups, a single tumor with a major
Table 5. Quantification of preneoplastic and carcinomatous lesions on the colonic mucosae of rats fed experimental diets over a period of 39 wk.

<table>
<thead>
<tr>
<th></th>
<th>−DCA</th>
<th>+DCA</th>
<th>2-way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Casein</td>
<td>HMF</td>
<td>Diet</td>
</tr>
<tr>
<td>Aberrant crypts</td>
<td>158 ± 15[b]</td>
<td>218 ± 32[b]</td>
<td>0.000</td>
</tr>
<tr>
<td>Aberrant crypts foci</td>
<td>36.6 ± 3.3[a]</td>
<td>44.5 ± 6.2[a]</td>
<td>0.000</td>
</tr>
<tr>
<td>Crypts/foci</td>
<td>4.3 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>0.055</td>
</tr>
</tbody>
</table>

These rats were the same as those used in Table 4. The quantification procedures for preneoplastic or carcinomatous lesions were detailed in Materials and Methods. Values are means ± SE (n = 5-10); those not sharing a common superscript in the same row are significantly different at p < 0.05.

# There is no significant difference between the DCA-fed casein group and DCA-fed HMF group at p < 0.05 (Fisher’s exact probability test).

* There is a significant difference between these two groups at p < 0.05 (Student’s t-test).

axis of 4.0 mm was found in the casein group (n = 10) but none were found in the HMF group (n = 5). The average number of tumor-bearing rats within each casein or HMF group was obtained as 3.0 ± 0.4 (n = 6) and 1.0 ± 0.0 (n = 3), respectively; the significance of difference between the two groups was assessed at p < 0.01 by Student’s t-test.

DISCUSSION

There are conflicting views on whether dietary soybean protein has a protective effect against chemically-induced tumorigenesis in the intestine. McIntosh et al. (16) have reported that dairy proteins such as milk whey and casein are more effective in suppressing the development of dimethylhydrazine-induced colonic tumors than soybean protein. As a possible explanation of the reason, they have referred to the following background: soybean protein relative to dairy proteins (a) stimulates cell proliferation by damaging the colonic epithelium, (b) brings fatty acids as well as bile acids much more into the colon by adsorption on unabsorbed ‘hydrophobic’ leavings, and (c) serves less as a precursor source for the synthesis of glutathione detoxifying or eliminating mutagens. On the contrary, many of the animal studies on experimental tumorigenesis are in favor of the view that soybean products possess anti-tumorigenic effects. In most cases, however, the tumor-suppressing effects were attributed to such concomitants as isoflavonoids, protease inhibitors and phenolic compounds rather than the soybean protein itself, in which the target organ is mainly the mammary gland. In this connection, the genistein, daidzein and total isoflavonoid contents in HMF were 0.07, 0.03 and 0.11%, respectively, on a dry weight basis (information from Research Institute of Fuji Oil Co.). Upon calculation, the HMF diet used in this experiment includes genistein at 144 mg/kg diet. According to a recent report, the administration of genistein at 150 mg/kg diet to AOM-treated rats caused a reduction in ACF of 34% at 4 wk (17). In our experiment, no significant difference in ACF at 12 or 39 wk was observed between DCA-unfed HMF and casein groups. There is little evidence to demonstrate that isoflavones such as genistein and daidzein can actually prevent the colon from tumorigenesis in vivo. It thus seems unreasonable to consider that such isoflavonoids occurring in HMF serve as major preventive factors against colonic tumorigenesis. Previous experimental results with highly purified proteins suggest that the lower sulfur-amino acid content in soybean protein, as compared to casein, is beneficial to the retardation of mammary tumor growth (18, 19), while the very amino acid composition of soybean protein is looked upon as a disadvantage in alleviating dimethylhydrazine-induced colonic tumors (16). On the assumption that an insufficient supply of sulfur-amino acids may affect chemically-induced tumorigenesis in the colon, HMF and casein diets were supplemented with dl-methionine so as to be exactly alike in sulfur amino acid content. Additionally, it had been confirmed by a preliminary experiment a few months earlier that the 14.7% casein diet ( +0.3% dl-Met) was almost equal in rat growth to the 20.6% HMF diet ( +0.4% dl-Met) under AOM-untreated conditions (data not shown). The difference in protein source content between these diets (i.e., about a quarter of HMF) was interpreted as dietary fiber in a broad sense mainly consisting of an indigestible or unabsorbed ‘resistant protein,’ so that cellulose powder was omitted from the HMF diet. Such a proteinous fiber left unabsorbed in the HMF digesta is far more hydrophobic than cellulose, being actually reflected in increased excretions of total lipids and steroids into the feces (Fig. 1 and Table 4). Alternatively, their
increments may have resulted in a lowering of the plasma cholesterol in the HMF groups. Even so, there remain risks of fatty acids and secondary bile acids occurring at high concentrations in the colon prior to their fecal excretion, as pointed out by McIntosh (16). It naturally follows that the safety of HMF from a tumorigenic point of view must be verified in actual experimenta- tion with laboratory animals.

Early in the 39th wk, one of the rats treated with AOM beforehand died from some unknown cause, although there was neither abnormality in appetite nor body weight until the day before death. The colon was immediately excised and examined for tumor development; however everything was in good order in the colon. Even though tumorigenesis was not directly responsible for the death, the duration of feeding was broken off at 39 wk in the fear of a further decrease in the finite population. ACF arising from AOM treatment are generically referred to as "soybean resistant proteins." "Soybean resistant protein" is the main body of the lucanic point of view must be verified in actual experi-

Acknowledgments

This study was financially supported by bounties from the Soy Protein Research Committee of Japan and by a Grant-in-Aid for Scientific Research (No. 09660142) from the Ministry of Education, Science, Sports and Culture of Japan.

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Antitumorigenicity of Soybean Resistant Protein


