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Effects of Dietary Fats and Soybean Protein on Azaserine-induced Pancreatic Carcinogenesis and Plasma Cholecystokinin in the Rat[1]

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ABSTRACT

Both dietary unsaturated fat and raw soybean products are known to enhance pancreatic carcinogenesis when fed during the postinitiation phase. A comparison of these two dietary components was made to evaluate the relative potency of each ingredient for enhancing pancreatic carcinogenesis and to determine if this enhancement was correlated with an increase in plasma cholecystokinin (CCK) levels. Male Wistar rats were initiated with a single dose of azaserine (30 mg/kg body weight) at 14 days of age. The rats were weaned to test diets formulated from purified ingredients. Dietary protein at 20% by weight was either casein or soy protein isolate (heat treated or raw). Corn oil was the unsaturated fat of major interest and it was fed at either 5 or 20% by weight. Pancreases were quantitatively evaluated for carcinogen-induced lesions at 2- and 4-month postinitiation. In a second experiment designed to closely mimic the above experiment, rats were implanted with cannulae which allowed plasma to be repetitively sampled over a 2.5-week period during which the test diets were fed. Plasma was collected both prior to introduction of the test diets and afterwards. Plasma CCK was measured by a specific radioimmunoassay. Both the 20% corn oil diet and the raw soy protein isolate diet enhanced pancreatic carcinogenesis. The effects of the raw soy protein isolate on the growth of the carcinogen-induced lesions were significantly greater than the effects of the 20% corn oil diet. Plasma CCK values were not elevated in the rats fed the 20% corn oil diet, but they were significantly elevated in the rats fed the raw soy protein isolate. Heat-treated soy protein isolate neither enhanced carcinogenesis nor elevated the plasma CCK level. This study demonstrates that certain plant proteins enhance the growth of carcinogen-induced pancreatic foci and that this effect is considerably greater than the enhancement by high levels of dietary unsaturated fat. Furthermore, the enhancement by the raw soy protein isolate may be mediated by CCK; but this does not appear to be the mechanism by which the unsaturated fat, corn oil, enhances pancreatic carcinogenesis.

INTRODUCTION

Pancreatic cancer is the fifth most common cause of death due to cancer; it is usually diagnosed late thus precluding effective treatment; and, with the exception of an association with the smoking of cigarettes, the etiology of this cancer is largely unknown (1). Using international epidemiological data of dietary fat intake and cancer mortality, a positive correlation of pancreatic cancer mortality with per capita fat consumption has been shown (2, 3). Experimental pancreatic carcinogenesis has provided strong support for an involvement of dietary fats in pancreatic carcinogenesis (4–6). Treatment with azaserine, a known pancreatic carcinogen for the rat, and the concurrent feeding of the UNSAT[4] diet, but not the SAT diet, enhanced the incidence and multiplicity of pancreatic cancer as compared to rats fed a control diet (AIN) containing 5% unsaturated fat (7). The effects on pancreatic cancers were similar in the groups fed either the SAT or the AIN diets, even though the total lipid contents of these two diets were very dissimilar. Subsequent studies in the rat (8) and the hamster (9) have shown that dietary unsaturated fat acts during the postinitiation phase of carcinogenesis. Recently a short-term, quantitative, rat-azaserine model has been developed which depends upon the identification and quantification of azaserine-induced, putative, preneoplastic foci of atypical acinar cells (10, 11). Using this model, we have shown that feeding the UNSAT diet as compared to the SAT diet increased the number of foci present and the tumor burden in the pancreas (11).

The feeding of a diet containing a high content of raw, full-fat soybean flour enhanced pancreatic carcinogenesis in rats when fed either concurrently with (12–15) or following (13) exposure to a known pancreatic carcinogen. In noncarcinogen-treated rats, the long-term feeding of soy products resulted in various hyperplastic lesions of the exocrine pancreas including adenomas and adenocarcinomas (16, 17). It is well established that the feeding of raw soy flour, particularly to chickens (18) and rats (19–22) leads to a rapid and dramatic enlargement of their pancreases. This is due to both hypertrophy and hyperplasia of the acinar cell component of the pancreas. These hypertrophic, hyperplastic, and carcinogenic effects are largely abolished by heat-treatment of the soy flour (17, 23). These growth stimulatory effects on the pancreas generally have been attributed to the proteinaceous TI content of the flour. It has been hypothesized that TI stimulates the release of CCK which is known to be trophic for the pancreas (17, 24). The research on soy flour and its effects on the rat pancreas has recently been reviewed (25).

These studies were undertaken for two reasons. First, raw, full-fat soy flour has been fed in a large number of experiments of pancreatic carcinogenesis, but as used the raw, full-fat soy flour not only contains TI but also approximately 20% unsaturated fat (26). Soy oil is highly unsaturated and of generally similar fatty acid composition (27) to corn oil, which at 20% in the diet enhances pancreatic tumorigenesis. Thus, we wished to compare the relative contributions of TI and an unsaturated fat to the postinitiation enhancement of pancreatic carcinogenesis. An abstract (28) and a preliminary note (13) of this portion of our work have been presented. Second, we wished to directly evaluate whether diets high in fat or soybean trypsin inhibitor increased plasma CCK levels and pancreatic organ weights. It is possible that enhanced pancreatic carcinogenesis by fat and soy protein isolate could result from similar mechanisms, namely, the trophic effects on the pancreas of increased CCK.

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[4] The abbreviations used are: UNSAT, modification of AIN diet with 20% unsaturated fat; AIN, American Institute of Nutrition purified, powdered diet having 5% unsaturated fat (29, 30); SAT, modification of AIN diet with 20% saturated fat; HSI and RSI, diets with heated and raw soy protein isolate, respectively, where the isolate replaced the casein of the AIN diet; TI, soybean trypsin inhibitor; CCK, cholecystokinin.

MATERIALS AND METHODS

Two experiments were performed. The first experiment evaluated the effects of several diets on the development of putative, preneoplastic foci in a well-characterized rat model of pancreatic carcinogenesis. The second experiment evaluated the effects of these same diets on the level of plasma CCK.

Diets. Composition of the powdered diets (Teklad, Inc., Madison, WI) that were fed to the rats in both the experiments is shown in Table 1. These diets were either the purified diet AIN (29, 30) or modifications of this diet. Both food and water were available *ad libitum*. All diets contained 20% protein as either casein or soy protein isolate. As fed, HSI contained 46 mg TI and RSI contained 600 mg TI per 100-g diet. The soy protein isolates were obtained from Dr. J. J. Rackis, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, IL. Extensive details of their preparation are published (31).

Pancreatic Tumorigenesis Model. Suckling male Wistar rats (Charles River Breeding Laboratories, Inc., Wilmington, MA) were injected i.p. at 14 days of age with a single dose of 30 mg azaserine (Calbiochem-Behring Corp., LaJolla, CA)/kg body weight as has been described (6). Rats were autopsied after 2 or 4 months of feeding the test diets. The entire pancreas was excised, fixed, and embedded by a standardized method for routine histology. The sections, stained with hematoxylin and eosin, were systematically examined by light microscopy (32). The quantitative analyses were limited to the splenic segment of the pancreases unless specifically stated otherwise.

Azaserine-induced lesions (henceforth called "foci") of acinar cells were identified and classified as acidophilic or basophilic in general accord with the criteria of Rao *et al.* (33) and Roebuck *et al.* (11). Details concerning the measurement of the focal transections are reported (11, 34). From the observed number and area of the focal transections, the mean number and mean size of the foci were determined by quantitative stereological methods (35). The details of the application to pancreatic foci have been published (11). In a few cases, limited to the two groups fed the raw soy protein isolate for 4 months, the pancreas sections contained so many and such large foci that the foci coalesced and the measurement of individual foci was not possible. In such cases, the point-counting method of Weibel (36) was used to determine the volume percentage of pancreas occupied by foci.

Surgical Preparation of the Rats. A chronically indwelling catheter was implanted in each of 33 male Wister rats using techniques that have been described in detail previously (37). In brief, a catheter was inserted into the left femoral artery and advanced into the descending aorta. The catheter was then tunneled s.c. to the dorsal side where it was exteriorized behind the neck. The catheter was filled with a 1:1 mixture of heparin (1000 U/ml) and 50% dextrose in water, and plugged with a stainless steel pin. Following surgery, the rats were monitored for at least 5 days before use in the following experiment.

Effects of Diet on Plasma CCK. A schematic of the experimental protocol is presented in Fig. 1. The protocol simulates that used for the carcinogenesis experiments but over a shorter time scale. The surgical procedures and the multiple blood samples dictated that we used rats of approximately 175 g at surgery. Hematocrits of selected rats were checked periodically to assure that anemia did not occur; we found no such evidence. All blood collections were made between 9:00 a.m. and 12:00 noon. The pin was removed and the contents of the cannula (approximately 0.1 ml) were withdrawn and discarded. Next, 0.3 ml of fresh blood was withdrawn to thoroughly rinse the cannula. The actual sample (0.4 ml) was then collected in a plastic tube containing EDTA. The initial 0.3 ml of blood was then returned, followed by 0.4 ml of 0.9% saline to replace the sample volume. Finally, the cannula was refilled with fresh heparin/dextrose and the pin reinserted. Following centrifugation, the plasma was transferred to another plastic tube (without EDTA), frozen immediately, and stored at −70°C.

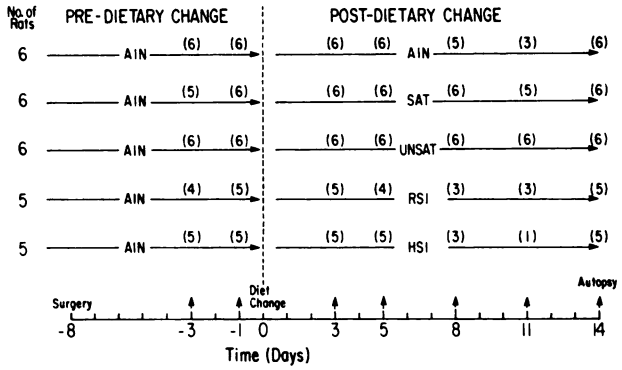

Fig. 1. Protocol for the evaluation of the effects of diet on plasma CCK levels. *Arrows above the time scale,* days on which plasma was collected; values in parentheses, number of rats from which blood was successfully collected; *horizontal arrows,* diets used in the control (predietary change) and experimental (postdietary change) periods.

Cholecystokinin Radioimmunoassay. The measurement of CCK in this study relied on the method of Izzo *et al.* (38) as previously described. The detection limit of this assay was 0.25 fmol of peptide in 50 μl of plasma (for CCK-8 this represents approximately 5.5 pg/ml of rat plasma). The radioimmunoassay was highly specific for sulfated forms of CCK: 30 pM of CCK-8 and 100 pM of CCK-33 displaced 50% of the radioligand, respectively, while gastrin demonstrated only slight cross-reactivity at concentrations greater than 300 nM.

Statistical Analyses. Differences between multiple groups were evaluated by analysis of variance, followed by a Neuman-Keuls test. When two cross comparisons could be made simultaneously, as with heated *versus* raw soy protein isolate and high *versus* low fat, two-way analysis of variance was utilized. For comparison between two groups, a *t* test was employed, or a paired *t* test when comparing paired group means.

Table 1 *Composition of the diets as percentage by weight*

All diets were stored at 4°C prior to feeding. The diets were fed *ad libitum* as powders.

Ingredient	AIN	SAT	UNSAT	RSI or HSI	RSI + UNSAT or HSI + UNSAT
Casein[a]	20.0	20.0	20.0		
Soy protein[a]				20.0	20.0
Methionine	0.3	0.3	0.3	0.3	0.3
Cornstarch	15.0	11.7	11.7	15.0	11.7
Sucrose	50.0	38.3	38.3	50.0	38.3
Cellulose	5.0	5.0	5.0	5.0	5.0
Unsaturated fat[b]	5.0	2.0	20.0	5.0	20.0
Saturated fat[c]		18.0			
Micronutrients[d]	4.7	4.7	4.7	4.7	4.7

[a] Protein levels are listed on an "as is" basis. Casein assayed to 87% protein and soy protein assayed at 82% protein.

[b] Corn oil was the unsaturated fat used in these experiments.

[c] Saturated fat was 18% hydrogenated coconut oil with 2% corn oil added to provide for the essential fatty acid requirements of the rats.

[d] Composition of the vitamin and mineral mixtures are those recommended for the AIN diet (29, 30).

RESULTS

In experiments to evaluate various diets on the development of azaserine-induced foci, autopsies were performed at 2 and 4 months into the postinitiation phase. The raw soy protein isolate at the levels which we fed did not inhibit the normal growth of the rats (Table 2). An increase in body weight was quite apparent in those groups fed the high fat diets for 4 months ($P < 0.01$). The pancreases of rats treated with azaserine have increased weights in part due to the azaserine-induced focal tissue. A small group of saline-treated rats held for 4 months (Table 2) revealed that the two groups fed the RSI also had significantly ($P < 0.001$) enlarged pancreases compared to the appropriate HSI group. The high fat diet did not result in pancreatic enlargement.

The major focus of this investigation is on the effects of diets on the growth of the azaserine-induced foci; however, for comparative purposes, pancreases of a few saline-injected (noninitiated) rats were thoroughly examined after feeding selected diets for 4 months postinjection (Table 3). Because so few foci were observed in these sections, the values in Table 3 are based

Table 2 *Body weights and pancreatic weights at 2- and 4-month postinitiation*

At 14 days of age rats were either initiated with a single dose of azaserine (30 mg/kg body weight) or injected with an equivalent volume of saline. They were weaned at 21 days of age and fed the test diets. At 2 and 4 months postinitiation rats were autopsied and pancreases excised. A description of the diets used is in Table 1.

	Saline treatment (4 months)			Azaserine treatment					
				2 months			4 months		
Diet	No. of rats	Body weight (g)	Pancreas weight (g)	No. of rats	Body weight (g)	Pancreas weight (g)	No. of rats	Body weight, (g)	Pancreas weight (g)
AIN	4	593 ± 21[a]	1.76 ± 0.12	0			7	610 ± 27	1.80 ± 0.09
SAT	0			0			8	648 ± 15	1.58 ± 0.06
UNSAT	5	689 ± 36[b]	1.79 ± 0.03	0			9	674 ± 26[b]	1.68 ± 0.06
RSI	5	535 ± 21	2.40 ± 0.14[c]	5	421 ± 16	2.61 ± 0.19[c]	10	537 ± 12	2.83 ± 0.14[c]
RSI + UNSAT	5	618 ± 22[b]	2.11 ± 0.04[c]	5	409 ± 17	2.04 ± 0.05[c]	10	579 ± 13[b]	3.32 ± 0.21[c]
HSI	0			5	432 ± 14	1.68 ± 0.04	10	539 ± 16	1.73 ± 0.05
HSI + UNSAT	0			5	463 ± 21	1.50 ± 0.99	10	627 ± 18[b]	1.68 ± 0.11

[a] Mean ± SE.
[b] Body weights of UNSAT groups significantly greater than their low fat paired diets ($P < 0.05$).
[c] Pancreas weights of RSI and RSI + UNSAT groups significantly greater than other groups ($P < 0.05$).

Table 3 *Pancreatic foci of saline-injected control rats at 4-month postinjection*

Acidophilic and basophilic foci of these saline control pancreases were identical in phenotype to the acidophilic and basophilic foci induced by azaserine. Regional differences within the pancreases were not apparent; thus, because the total number of foci per pancreas was so small in these control rats we present data based upon the combined splenic and duodenal regions. The focal data in Tables 4 and 5 are based on only the counts from the splenic region of the pancreas. Since the splenic and duodenal regions are of approximately the same size in our tissue sections, a more direct comparison of the "spontaneous" foci and the effects of the azaserine are seen if the number of foci in this table are reduced by half. Because there were so few foci, the usual quantitative and statistical analysis of the foci were not undertaken.

	Acidophilic foci				Basophilic foci			
Diet (no. of rats)	Total no. of foci observed	No./cm²	Transectional area (mm² × 100)	Volume of % of pancreas	Total no. of foci observed	No./cm²	Transectional area (mm² × 100)	Volume as % of pancreas
AIN (4)	0	0		0	0	0		0
UNSAT (5)	3	0.14 ± 0.06[a]	22.1 ± 19.1	0.033 ± 0.030	3	0.14 ± 0.06	5.05 ± 2.21	0.007 ± 0.004
RSI (5)	7	0.25 ± 0.07	13.7 ± 4.2	0.028 ± 0.008	3	0.15 ± 0.15	4.13 ± 0.01	0.006 ± 0.006
RSI + UNSAT (5)	14	0.56 ± 0.21	25.2 ± 10.9	0.123 ± 0.066	7	0.31 ± 0.17	2.38 ± 0.53	0.009 ± 0.005

[a] Means ± SE.

upon counts from both the duodenal and splenic regions; whereas, in the subsequent tables the counts are based only upon measurements from the splenic region. Relative to the azaserine-initiated pancreas, extremely few foci were counted in the saline controls and there were not enough rats per group nor enough foci per rat to statistically evaluate the effects.

At both 2- and 4-month postinitiation, the pancreases of the azaserine-treated rats contained both acidophilic and basophilic foci. The acidophilic population clearly predominated, being approximately 10 times more numerous. A detailed analysis of the basophilic foci revealed no indication of focal growth in response to dietary treatments. Therefore, the subsequent results are limited to the acidophilic population.

The effects of feeding the soy protein isolate and high unsaturated fat-containing diets for 2 months are presented in Table 4. From the two-dimensional data (number of focal transections per sq. cm), one would conclude that there were more foci in the two groups fed the raw soy protein isolate than in the two groups fed the heated soy protein isolate ($P < 0.01$). However, applying quantitative stereological equations to achieve a three-dimensional approximation of focal number and size, we observe that all 4 groups had a similar number of foci per cu. cm of splenic pancreas ($P > 0.05$). This serves as an example of the bias inherent in the observed transectional data. The explanation for the bias is that small foci are less likely to be within a tissue section and are therefore less likely to be observed and counted. The addition of fat to either of the soy diets did not result in more or larger foci. The volume percentage takes into account the effects of both the number of foci and their size. This can be thought of as the tumor or focal burden within the pancreas. The tumor burden was significantly enhanced by the raw soy protein isolate ($P < 0.05$), but not by the 20% unsaturated fat.

The effects of feeding test diets for the 4-month postinitiation

Table 4 *Postinitiational effects of several diets on azaserine-induced, acidophilic foci of rat pancreas: 2 months*

Rats were treated at 14 days of age with azaserine (30 mg/kg) and weaned at 21 days to the diets listed below. Diets were fed *ad libitum* for 2 months prior to autopsy. The splenic or tail portion of all the pancreases were examined by light microscopy for foci. Both acidophilic and basophilic phenotypes were identified by criteria previously described (11, 33). Only the acidophilic foci are presented as so few basophilic foci (mean of <4 foci/splenic section) were observed in these rats. The quantitative analysis of the focal transections was according to the method of Pugh *et al.* (35) as adopted for pancreas (11).

		Observed transectional data of foci		Calculated volumetric data of foci		
Diet (no. of rats)	Mean no. observed	No/cm²	Mean area (mm² × 100)	No/cm²	Mean diameter (μm)	Volume as % of pancreas
RSI (5)	106 ± 14[a]	34.6 ± 2.9	21.8 ± 5.4	701 ± 89	516 ± 61[b]	7.57 ± 1.84[b]
RSI + UNSAT (5)	106 ± 10	41.3 ± 2.4	23.0 ± 3.2	842 ± 58	497 ± 32[b]	9.69 ± 1.66[b]
HSI (5)	46 ± 9	17.1 ± 2.5	6.4 ± 0.8	657 ± 127	275 ± 29	1.12 ± 0.24
HSI + UNSAT (5)	44 ± 11	22.2 ± 4.8	4.8 ± 0.9	957 ± 127	222 ± 30	1.21 ± 0.44

[a] Mean ± SE.
[b] Different from other two groups ($P < 0.05$).

Table 5 *Postinitiational effects of several diets on azaserine-induced, acidophilic foci of rat pancreas: 4 months*

All methods are the same as described in Table 4 except these rats were fed the postinitiation diets for 4 months instead of 2 months.

	Observed transectional data of foci			Calculated volumetric data of foci		
Diet (no. of rats)	Mean no. observed	No/cm²	Mean area (mm² × 100)	No/cm³	Mean diameter (μm)	Volume as % of pancreas
AIN (7)	55 ± 9[a]	25.5 ± 3.5	15.0 ± 1.8	558 ± 61	448 ± 28	4.06 ± 0.82
SAT (8)	21 ± 4	15.1 ± 2.8	7.3 ± 0.8	470 ± 72	302 ± 22	1.24 ± 0.29
UNSAT (9)	52 ± 8	34.3 ± 4.5	23.1 ± 2.9	727 ± 81	469 ± 34	8.75 ± 2.04[b]
RSI (10)[c]	107 ± 13	37.3 ± 1.4	41.9 ± 5.8	542 ± 34	702 ± 38	19.46 ± 4.27[d]
RSI + UNSAT (10)[c]	78 ± 12	33.4 ± 2.3	72.5 ± 17.3	406 ± 48	878 ± 118	32.15 ± 3.87[b, d]
HSI (10)	55 ± 9	28.3 ± 3.3	14.6 ± 1.7	607 ± 53	460 ± 27	4.44 ± 0.79
HSI + UNSAT (10)	55 ± 9	28.2 ± 3.7	15.1 ± 3.0	665 ± 52	415 ± 32	5.10 ± 1.65[b]

[a] Mean ± SE.
[b] Addition of UNSAT to the base diet increased the volume percentage ($P < 0.05$).
[c] Individual foci in some pancreases within these two groups were so large and numerous that they could not be accurately measured. Calculation of the number and diameter of foci in the RSI group is based upon only nine rats and in the RSI + UNSAT the basis is only five rats. The calculation of volume as percentage of pancreas is unaffected by problems of distinguishing individual foci, one from another.
[d] RSI increased volume percentage ($P < 0.05$).

period are presented in Table 5. The number and size of foci in the RSI and RSI + UNSAT groups were so large for certain rats that many individual foci coalesced and could not be distinguished, one from another. For this reason, certain data from some rats could not be included (see Table 5, *footnote b* for details). Again, as seen for the 2-month postinitiation data, there were more foci observed in pancreases of rats fed the raw soy protein isolate compared to the heated product. As before, a look at the number per cu. cm reveals that the two-dimensional data are not a good indicator of three-dimensional actualities. Unfortunately, the calculated volumetric data for the RSI groups appear lower than they actually are, because the most responsive rats with the large coalesced foci could not be included. This may explain why the number of foci per cu. cm for the RSI + UNSAT group (with 5 of 10 rats excluded) was significantly lower than the other groups. In spite of this problem, the foci of the two groups fed raw soy isolate (Table 5) were significantly larger than the foci of the two heated soy isolate groups ($P < 0.05$).

In those four groups fed the soy protein isolate (Table 5), there was not a significant effect of the high unsaturated fat on the size of the foci. Again, some rats could not be included in the data for these groups. The calculation of focal volume as percentage of pancreas is unaffected by the inability to measure individual foci, thus, the mean values are based on all the rats in a group. The volume percentage indicates the very large magnitude of the effect of raw soy protein isolate on the growth of the foci. An analysis of the groups fed raw soy protein isolate for 4 months reveals a significant effect of the high fat on the volume percentage ($P < 0.01$).

As a further indication of the magnitude of the effect due to the high fat and the soy protein isolates, three groups of rats fed casein-based diets instead of the soy protein isolate diets were evaluated (Table 5: AIN, SAT, and UNSAT). The postinitiational effects of feeding the AIN and the HSI diets, differing only in the source of dietary protein, were similar. The number and size of pancreatic foci among the three groups fed the casein-based diets were not significantly different, however, the UNSAT group volume percentage was larger ($P < 0.05$) compared to either the AIN or the SAT group.

A comparison of the data in Tables 4 and 5 leads to some important observations. For each specific diet, there were fewer foci (number per cu. cm) at 4-month than at 2-month postinitiation. The explanation for this observation is not readily apparent. However, the mean diameter and volume percentage of foci increased from 2 to 4 months. At 2-month postinitiation, the foci of the two groups fed the raw soy protein isolate were significantly larger (mean diameter or volume percentage) than the foci of the two groups fed the heated soy isolate. From 2 to 4 months, the growth of the foci in all groups appears to be at the same rate. The mean volume percentage for each group increased 3–4-fold during this time period, while the mean diameter increased 1.5–2-fold. Thus, it appears as if the most dramatic effects of the soy protein isolates occur within the first 2 months of the postinitiation phase. Furthermore, the focal growth-stimulating effects of the raw soy isolate are considerably greater than the effects of feeding a high unsaturated fat diet.

In an experiment designed to closely approximate the pancreatic carcinogenesis experiments reported above, measurements of plasma CCK have been made. The protocol for these experiments are outlined in Fig. 1 and the plasma CCK values are tabulated in Table 6. For presentation purposes, the effects of dietary change on plasma CCK have been divided and tabulated into early effects (week 1) and later effects (week 2). The plasma CCK values prior to the dietary change (days −3 and −1) were 38.4 ± 1.8 and 38.0 ± 1.4, respectively. During this time period, all rats were fed the AIN control diet. The one group that continued with this diet showed a gradual decline in the plasma CCK level over the 2-week postdietary change period. Similar trends were observed for the SAT and UNSAT groups. At autopsy, the weights of pancreases in these three groups were very similar.

A comparison of the AIN group with the two soy isolate groups is important. These three groups differed only in the source of protein. The plasma CCK was higher in the HSI group than the AIN group during both week 1 ($P < 0.05$) and

Table 6 *Effects of various diets on plasma CCK levels*

Details of the experimental protocol are outlined in Fig. 1. At the two control times of days −3 and −1, the mean plasma CCK values (pg/ml) were 38.4 ± 1.8 and 38.0 ± 1.4, respectively. At autopsy trunk blood was collected from those rats whose cannula was no longer patent. There was no apparent difference in CCK values from cannula or trunk blood. Pancreatic weights are from the autopsies at the termination of the experiment.

Postdietary change	Plasma CCK (pg/ml)		Pancreas weights (g)
	Week 1	Week 2	
SAT	33.0 ± 3.1[a]	23.5 ± 2.6	1.15 ± 0.03
UNSAT	36.0 ± 3.5	28.7 ± 2.7	1.11 ± 0.06
AIN	32.2 ± 1.6	27.5 ± 2.2	1.16 ± 0.05
RSI	45.3 ± 1.9[b]	50.1 ± 5.1[c]	1.79 ± 0.06[d]
HSI	39.8 ± 2.8[b]	37.4 ± 9.8	1.13 ± 0.05

[a] Mean ± SE.
[b] During week 1, CCK values for both RSI and HSI differed from AIN ($P < 0.05$).
[c] During week 2, CCK values for RSI differed from AIN ($P < 0.05$).
[d] Differed from all other pancreatic weights ($P < 0.05$).

week 2 (not significant). This increase did not, however, result in an increase in pancreatic weight. Of all the groups examined, the only one to show a consistent, positive increase in plasma CCK was the RSI group. During both weeks 1 and 2, the CCK values of the RSI group were greater than the AIN, SAT, or UNSAT groups. Not only were the plasma CCK values consistently high in the RSI group, but the mean pancreatic weights were greater ($P < 0.05$) than for the pancreases of any of the other groups.

DISCUSSION

Two issues of major importance are addressed in this study; namely, the relative contribution by dietary unsaturated fat *versus* the raw soy protein isolate on the enhancement of pancreatic carcinogenesis *and* the role of CCK in mediating this enhancement. These two issues will be addressed below. Several secondary issues were important to the success of the study and must be considered. First, these diets and the experimental protocols did not inhibit the normal rate of growth (data not shown) or suppress the final body weights attained by these rats (Table 2). Previous experiments have shown that decreased growth by caloric restriction (7, 8, 39) or toxicity (40) inhibits pancreatric carcinogenesis, and food deprivation is known to suppress plasma CCK activity (41). Second, foci were observed in those rats not treated with azaserine (Table 3); however, the effects of the carcinogen azaserine were overwhelmingly larger. Therefore, the contribution by the few "spontaneous" foci is expected to be negligible. In experiments of such short duration and with such few saline-injected rats (Table 3), it is not possible to determine the relative contribution by fat or the soy protein isolates to the "spontaneous" tumor burden. Others have noted an increased incidence of foci with increasing age of control rats maintained over 2 years (42, 43). The effects of feeding raw, full-fat soy flour for 2 years is known to result in a high incidence of "spontaneous" acinar cell tumors including adenocarcinomas (17). At present, it is not possible to determine if soy products contain a carcinogen or if they are simply enhancing the development of tumors initiated by other factors of either endogenous or exogenous origin. Third, the contribution of the basophilic focal population to either the number of foci or the focal burden was very small in this study. Evidence to date indicates that the basophilic focal population has little growth potential as compared to the acidophilic foci (11, 33, 44). For this reason, the omission of the few basophilic foci would not be expected to alter the conclusions drawn from this study.

From these studies, it is quite obvious that soy protein, as opposed to unsaturated fat, is the dietary component largely responsible for the enhancement of pancreatic carcinogenesis in those initial experiments in which raw soy flour was fed. The effects of feeding the raw soy protein isolate on carcinogenesis appear to be several fold greater than the effects of feeding a high unsaturated fat diet. But, it is not possible from these experiments to assign quantitative values to the magnitude of the effects of these two major dietary components. Before attempting this, two major factors will have to be resolved. First, the response by the carcinogen-initiated rat pancreas to various levels of both total dietary fat and the unsaturated fat content will have to be delineated. Additionally, the response of this model to other levels of raw soy protein isolate is not known. Second, the possible interactions between fat content and soy protein would have to be described as well as any interaction with other dietary factors. From the results in Tables 4 and 5, one cannot clearly determine if an interaction does or does not exist between the dietary fat and the raw soy protein isolate. There are some studies to indicate that the level of dietary protein is important in the response of the pancreas to enzyme secretion and the hemostatic control of plasma CCK (45, 46). For this reason, it is important to make comparisons between groups fed protein from the same animal or plant source as well as to evaluate the effects of various levels of dietary protein.

An interesting observation from these studies is that the effects of raw soy protein isolate occur largely during the first 2 months and not during the last 2 months of the postinitiation phase of carcinogenesis. Additionally, the effects of the raw soy protein isolate during this early period of the postinitiation phase are to increase the size of the foci and not to increase the number of foci that emerge from the initiated pancreas. One explanation that must be experimentally confirmed is that the raw soy isolate causes a transient increase in the plasma CCK levels that lasts less than the first 2 months of the postinitiation phase. This certainly appears to be the response of plasma CCK to variations in the levels of dietary protein (45, 46).

A correlation of the enhanced growth of carcinogen-induced foci with elevated plasma CCK levels and with increased pancreatic weight only holds for the group of rats fed the RSI diet (Table 6). The rats fed the UNSAT diet showed enhanced tumorigenesis, albeit slight, but not elevated pancreatic weight or increased plasma CCK levels. This is evidence that CCK does not mediate the enhancement of pancreatic carcinogenesis by the dietary unsaturated fat, corn oil. In a previous study in which rats were maintained for a year, saline-treated controls fed the UNSAT diet did not have elevated pancreatic weights as compared to AIN fed rats (47). We cannot, however, be absolutely certain that CCK is not involved in the enhancement by unsaturated fats. CCK appears to evoke hypertrophy and hyperplasia of the exocrine pancreas and appears indistinguishable from the effects of raw soybean protein (24, 25). It also remains to be determined if CCK selectively stimulates growth of the carcinogen-induced foci. It is known that the UNSAT diet increases the growth of individual foci, but not the pancreas in general (35, 48).

In summary, the protein component of raw, full-fat soybean flour contributes to the enhanced azaserine-induced pancreatic carcinogenesis to a far greater extent than the high content of unsaturated fat of this flour. However, it would be expected that the effects of enhancement by the unsaturated fat would remain after heat treatment of any soybean products. These effects of the raw soy protein isolate on the pancreas appear to be mediated through an elevation in the plasma CCK; whereas, the effects of the high levels of dietary corn oil are probably not mediated through this mechanism.

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