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Effects of Soybean Flour on the Pancreas of Rats

by Eileen E. McGuinness,* Reginald G. H. Morgan† and Kenneth G. Wormsley*

We have reviewed the growth-promoting and carcinogenic effects of feeding raw soya flour to rats. If the raw soya flour-containing diets are fed for more than a year, about 10% of the animals develop pancreatic cancer. In addition, feeding raw soya flour markedly potentiates the action of even subthreshold amounts of pancreatic carcinogens. The raw soya flour therefore acts as a potent promoter, as well as a weak carcinogen. In view of this promotion, the rat fed raw soya flour is a sensitive model for screening pancreatic carcinogens.

It is not known whether the human pancreas responds to dietary trypsin inhibitors in a manner similar to the rat. However, in view of the use of soya-based products in human nutrition—especially in infant foods—we urge that the effect of all soya-based products intended for human use be tested on the rat pancreas in long-term feeding studies, combined with subthreshold doses of azaserine to highlight any promoting activity of the product. It seems probable that if a product exerts no effect on the rat pancreas, the human pancreas will also be spared from noxious effects.

Morphological Aspects

Macroscopic Features

Raw soya flour-containing diets have been shown to produce enlargement of the rat pancreas after administration for as little as 10 to 20 days (*1*). The pancreatic enlargement has been shown to reflect both hypertrophy (defined as increase in cellular size and amount of protein per unit of DNA) (*2*) and also hyperplasia (increase in the amount of DNA and number of cells in the gland) (*3*). We have shown that the pancreatic growth-promoting effects continue, if the administration of raw soya flour is maintained, but becomes progressively more marked in an exponential manner at some time between 30 and 60 weeks of feeding raw soya flour-containing diets (*4*).

During the period of pancreatic enlargement, the gland changes color to become dark red, reflecting increased vascularity and blood flow, as demonstrated by use of microspheres (unpublished results).

Some time between 24 and 30 weeks after the start of raw soya flour-containing diets, small greyish patches become visible on the surface of the gland. Subsequently, overt nodules appear and increase in size throughout the gland as the duration of exposure to the raw soya flour increases (*4*). The increased size of the nodules correlates with the exponential phase of increase in size and weight of the gland.

After administration of raw soya flour for more than 60 weeks, the pancreas becomes progressively more likely to develop carcinomatous change (*4*). About half the carcinomas spread to involve the liver, some clearly as a result of metastases.

Ten to fifteen percent of rats develop pancreatic cancer, if the rats are continuously fed raw soya flour in amounts ranging from 5 to 100% of the total protein of the diet (with unicellular protein making up the remainder). The proportion of rats developing pancreatic cancer is greater (up to 60%) in rats fed 100% raw soya flour for only 2 days each week for 60 weeks or more, with non-soya-containing chow for the remaining 5 days each week (*4*). All these values of incidence of pancreatic cancer are significantly greater than the maximal value of about 1% of "spontaneous" pancreatic neoplasms recorded in the literature (*5*), particularly bearing in mind that this value has been obtained from observations on rats fed "routine" diets, which have probably contained soya flour.

Our rats receiving diets free from soya flour have not developed abnormal foci, nodules, or pancreatic cancer. Similarly, rats receiving heated soya flour do not show abnormal increase in pancreatic weight during feeding for 2 years (*4*). Although a few microscopic and macroscopic nodules developed after feeding heated

*Department of Therapeutics, University of Dundee, Dundee DDI 9SY Scotland.
†Department of Physiology, University of Western Australia, Nedlands, W. Australia.

soya flour for 60 weeks or more, no animal fed heated soya flour developed pancreatic cancer (*4*).

Microscopic Appearances

Within 1 to 2 days after starting a diet containing raw soya flour, the rat pancreas shows patchy necrosis of isolated pancreatic acinar cells and acini, but this damage is rapidly resolved. If the animals continue to receive raw soya flour, further morphological changes occur, including enlargement of cells and acini, with increased zymogen content (*6*). These changes correlate with the growth of the gland and with the increased secretory response to stimulants of the pancreas of these animals (*7*).

After feeding raw soya flour-containing diets for 16 to 24 weeks, a few microscopic foci can be found scattered throughout the pancreas. The number and size of the nodules increases with the duration of feeding (*4*). The microscopic nodules are initially composed of acinar cells, containing zymogen granules, with more eosinophilic cytoplasm than the surrounding unaffected acinar tissue. The nuclei are larger and less dense and mitotic figures are much more common than in the surrounding, apparently normal, acinar tissue. When the nodules become larger, the acinar cells often appear to be arranged in tubular fashion. The surrounding pancreatic cells may appear to be compressed. The nodules become well defined and, after about one year of feeding raw soya flour, become separated from the surrounding pancreatic cells by a fibrous capsule. Islets of Langerhans are not seen within the nodules.

After a year or more of raw soya flour-containing diets, a part of the nodule may dedifferentiate with loss of zymogen granules and cytoplasm and crowding of nuclei. The appearances may suggest the development of a nodule within a nodule. Ultimately, the nuclei and cells become pleomorphic and invasion of the capsule or spread outside the capsule indicates overtly malignant transformation.

When azaserine is administered to rats fed raw soya flour, a higher yield of nodules and more rapid progression to carcinoma occurs (see below). In these animals, the microscopic appearances of the cancers range from well-differentiated acinar cell neoplasms (44%) to papillary (26%) or undifferentiated (30%) carcinomas. The metastatic lesions in the liver show a similar range of appearances, with well-differentiated lesions predominant.

Reversibility of Morphological Changes

The pancreatic weights, protein content and morphological appearances revert to normal when rats are fed raw soya flour for 5 days (*8*), 4 weeks (*9*) and 6 months (unpublished observations) and then receive non-soya-containing diets.

If rats are fed raw soya flour for as long as 6 months and the diet is then changed to non-soya-containing chow, nodules are not found at death a year or more later, so that histologically the pancreas of these rats resembles the morphological appearances of the pancreas of animals fed non-soya-containing diets throughout life. It seems that nodule formation can be reversed and, indeed, the development of pancreatic cancer prevented since even continuing azaserine does not result in pancreatic cancer in animals in which raw soya flour is discontinued after 12 weeks (*10*). Administration of raw soya flour for longer than 6 months results in irreversible nodule formation and progression to pancreatic carcinoma, despite change to non-soya flour-containing diets (unpublished observations).

Mechanisms of Promotion of Pancreatic Growth

The mechanisms whereby raw soya flour stimulates pancreatic growth and neoplastic transformation have not been defined. However, it has been proposed that raw soya flour, which contains protease inhibitors (*1,11*), acts on the pancreas indirectly by inhibiting trypsin within the lumen of the small intestine (*12*). Intraluminal trypsin is considered to inhibit pancreatic secretion by inhibiting the release of a stimulant hormone such as cholecystokinin by a "negative feedback" mechanism (*13,14*). As a consequence of tryptic inhibition by soybean trypsin inhibitor, there is therefore unfettered release of the pancreatic stimulant hormone (*15*) from the small intestinal mucosa. Administration of raw soya flour to rats does result in marked increase in the concentration of circulating cholecystokinin (*16*), and cholecystokinin has been shown to stimulate pancreatic hypertrophy and hyperplasia in rats (*17,18*). It has therefore been concluded that continuous release of large amounts of cholecystokinin during feeding of raw soya flour is responsible for the pancreatic growth.

However, other factors in raw soya flour are probably also involved in raw soya flour-induced pancreatic growth (*19*), since it has been calculated that as little as 40% of the enlargement of the rat pancreas produced by the ingestion of raw soybeans is accounted for by the trypsin inhibitor (*20*). The remainder may, in part, reflect the presence of undenatured soybean protein, which can itself bind trypsin. In addition, free fatty acids can produce trypsin inhibition (*21*), a finding which may explain the "promoting" effects both of heated soya flour (*22*) and high fat diets (*23,24*) on pancreatic carcinogenesis in rats.

Other mechanisms may also be operative. For example, soya bean flour adsorbs bile salts (*25*). Since cholestyramine, which adsorbs bile salts, also causes pancreatic hypertrophy and hyperplasia in rats (*26*), deficiency of intestinal intraluminal bile salts may be involved in the stimulation of pancreatic growth, either

directly or indirectly by limiting the absorption of free fatty acids from the intestinal lumen.

Cellular Kinetic Studies

DNA and RNA Content

Within a day of starting administration of soybean trypsin inhibitor, an increase occurs in the total pancreatic RNA and protein content of the neonatal rat (*27*). An increase in pancreatic DNA content occurs as early as 48 hr in the neonatal rats (*27*), but we have found that in young adult animals, total pancreatic DNA is not significantly increased until between the first and second week after starting to feed raw soya flour. Pancreatic DNA content then increases to plateau levels after 4 to 8 weeks of the raw soya flour diet and remains significantly raised as long as the animals are kept on the diet (*3*). However, these indices of growth have only been studied systematically during 36 weeks of raw soya flour-containing diets, and it is therefore not known whether DNA content rises exponentially, as does pancreatic weight, after this time. Pancreatic weight and DNA content increase only slowly and only in parallel with body weight between 8 and 36 weeks after start of the raw soya diet. It has been shown that the newborn rat is particularly sensitive to the trophic effects of raw soya flour on the pancreas (*27*), and the cessation of growth in older rats might therefore be attributed to failure of the mature rat pancreas to respond to the growth stimulation provided by feeding raw soya flour. However, feeding raw soya flour to 1-year-old rats previously fed non-soya-containing chow resulted in a trophic response at least as vigorous as that seen in young animals, with an increase in DNA content similar to the 140% increase reported in the young adult animals (*3*). The leveling off of growth and DNA content of the pancreas after 4 to 8 weeks of the raw soya flour diet is thus not caused by the aging of the rats. The factors involved in the blunting of the response to the (presumably) continued trophic stimulus have not been further defined.

An increase in total pancreatic DNA is usually interpreted as indicating hyperplasia (increased number of cells). This interpretation is correct only if the DNA content of individual cells does not change. In rats fed raw soya flour for prolonged periods, increasing variability in nuclear size and staining occurs. It is possible, therefore, that some of the changes in DNA content may be caused by changes in ploidy of a population of acinar cells. Recent measurement of nuclear DNA in acinar cells isolated from rats fed raw soya flour indicates that there is indeed an increased percentage of tetraploid cells in these animals (unpublished observations).

The pancreatic content of DNA and RNA remains significantly increased during feeding of raw soya flour. After feeding raw soya flour for four weeks, DNA content is increased by one-third compared with control rats and pancreatic weight, RNA and protein content are double the control values of animals fed non-soya diets. If the diet is then changed to heated soya flour or non-soya-containing chow, the DNA content reverts to control values within 48 hr, while pancreatic weight, RNA and protein content return to normal values within 1 week (*9*). This rapid involution is associated with surprisingly little morphological evidence of tissue destruction, though patchy apoptotic changes are seen. Animals undergoing this reversion to normal morphological appearances never seem distressed and no deaths have occurred in 50 animals during change to a non-soya-containing diet.

Indices of Cellular Replication

When rats are fed raw soya flour, pancreatic cell turnover is increased for the first 8 weeks of the diet, as shown by the incorporation of ^{3}H-thymidine into pancreatic DNA both *in vivo* (*28*) (unpublished observations) and *in vitro* (*29*). No change is seen within 24 hr of starting to feed raw soya flour, but a marked stimulation of DNA synthesis then occurs, peaking at about ten times that seen in control animals, 40 to 48 hr after starting the diet. The synthesis of DNA then decreases to control values after 5 to 7 days, before rising again to a broad peak of increased DNA synthesis, about five times that found in control animals, between $1^1/_2$ and 3 weeks after starting the raw soya diet. The synthesis of DNA then slowly declines, to reach control values after about 8 weeks and thereafter remains at control levels (Fig. 1).

Autoradiographic studies show that both acinar and centroacinar cells are labeled during the early peak, with acinar cells responding first. It appears, therefore, that at least some of the new cells produced as a result of feeding raw soya flour are derived from apparently mature acinar cells, but the relative contribution from

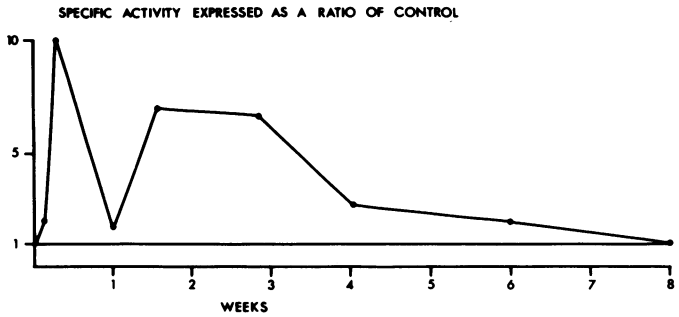

FIGURE 1. Specific activity of pancreatic DNA after 30 min of *in vitro* incubation with ^{3}H-thymidine. The activity in animals fed raw soya flour is expressed as a ratio of the activity in control animals fed heated soya flour (trypsin inhibitor inactivated) for the same period. Results are the mean of three assays on each of four or eight rats (*29*).

this source and from the centroacinar or duct cells has not yet been defined. During the later peak, duct cell labeling predominates. It has been suggested that the uncommitted pancreatic stem cell is morphologically similar to the centroacinar cell, so that these labeled cells may be the precursor of both duct and acinar cells in the enlarged gland (*30*).

Both the *in vivo* and *in vitro* studies show that after 8 weeks of raw soya flour diet, cell turnover has returned to values not greater than those observed in rats fed non-soya chow or heated soya flour. The latter finding is in accord with the observation that in these animals, mitotic activity is very low in apparently normal pancreatic tissue surrounding the hyperplastic foci. However, mitoses are common in the developing foci and this proliferative activity increases with the length of exposure to raw soya flour and the progression of the nodules. Cellular kinetics in the foci have not been systematically investigated, but we have demonstrated increased cell turnover in nodules in *in vitro* studies, during which the incorporation of ^{3}H-thymidine into DNA was measured in enucleated nodules and in the surrounding tissue from rats fed raw soya flour for 52 weeks. In the three animals studied, the specific activity of DNA from the nodules was 12, 6 and 10 times that of the surrounding tissue (*29*), indicating a marked increase in the rate of cell turnover in the nodules, compared with the surrounding normal tissue.

Effect of Additional Carcinogens

It has previously been reported (*31*) that replicating tissues are more sensitive to carcinogens than cells which are kinetically inert. We have therefore tested the effect of administering exogenous carcinogens to rats fed soya-derived and control diets.

Azaserine (*O*-diazoacetyl-L-serine), a derivative of serine (*32*) obtained from *Streptomyces fragilis*, has been shown to produce persistent DNA damage (*33*), nodule formation and, after administration for a year or more, pancreatic cancer in rats (*34*). We have shown rapid and marked potentiation between a diet containing raw soya flour and azaserine in as little as 24 weeks (*35*). While raw soya flour diets and azaserine singly produced only a few microscopic pancreatic nodules in a few rats during this period, the combined administration of a raw soya flour-containing diet and azaserine resulted in the development of multiple nodules in all animals (*35*). Subsequent studies showed that azaserine, in a dose of 5 mg/kg body weight each week, given intraperitoneally for 60 weeks, produced a few microscopic and macroscopic nodules in the rat pancreas but never resulted in pancreatic cancer if the rats were fed non-soya-containing diets (prepared with monocellular (yeast) protein as the source of protein). Azaserine (5 mg/kg) was therefore subcarcinogenic for the rat pancreas (*22*). Nevertheless, the administration of this subthreshold dose of azaserine induced pancreatic cancer in more than 60% of rats fed raw soya flour for more than 60 weeks (*10*). The potentiating effects of raw soya flour on the development of azaserine-induced pancreatic cancer also occurred when the raw soya flour was administered only after the end of a 3-week course of 5 mg/kg-week azaserine (unpublished) but if, alternatively, raw soya flour-containing diets were given for only 12 weeks, no pancreatic cancer developed in the rats given azaserine for as long as 60 weeks (*10*). A higher dose (15 mg/kg each week) of azaserine has produced pancreatic cancer in our rats, irrespective of diet (unpublished). Previous reports that 5 mg/kg each week of azaserine produced cancer (*34*) in rats fed commercial chow may reflect strain differences of may be attributable to different composition of the diet fed to the rats, since commercial rat chows have contained considerable amounts of soya proteins.

We have also studied the effect of a nitrosamine derivative, di(2-hydroxypropyl)nitrosamine, on the development of pancreatic cancer in rats fed raw soya flour (*36*). This nitrosamine had been reported to be only weakly carcinogenic for the pancreas of rats (*37*). We confirmed that when the nitrosamine was administered to rats receiving non-soya diets, no pancreatic cancer developed while pancreatic cancer did occur in treated animals fed raw soya flour (*36*). We concluded that, as with azaserine, the feeding of raw soya flour potentiated the effects on the rat pancreas of an exogenous carcinogen.

While no rats fed non-soya-containing diets and given subthreshold doses of azaserine developed more than a few microscopic nodules (*22*), the administration of azaserine to rats fed heated soya flour resulted in the development of pancreatic cancer in nearly 40% of animals (*22*). Heated soya flour thus also potentiates the carcinogenic effects of azaserine. It is possible that the fat content of our preparation of heated flour has been responsible, in part, for this potentiation, since it has subsequently been shown that 20% unsaturated fat in the diet of rats receiving azaserine increases the incidence of pancreatic cancer (*23,24*).

A further example of potentiation which is, however, rather more difficult to interpret comprises the finding that 60% of rats which survived an attack of ethionine-induced pancreatitis and were subsequently fed raw soya flour-containing diet developed pancreatic cancer. The combination of ethionine pancreatitis and raw soya flour diet therefore potentiated in a manner similar to the combination of azaserine and raw soya flour (*38*). In this case, ethionine may act as a pancreatic carcinogen —as it has been shown to act as a hepatic carcinogen (*39*)—with the production of initiated cells either as a direct result of the ethionine or during the hyperplastic response to pancreatic damage.

Implications for Evaluation of Pancreatic Carcinogenesis

Processes Involved in Carcinogenesis

It seems probable that a chemical such as azaserine is a typical genotoxic pancreatic carcinogen, which is capable of initiating carcinogenesis when administered in subthreshold doses but also of producing overt pancreatic cancer when given in larger doses. Raw soya flour promotes the pancreatic carcinogenic effects of azaserine, an effect best illustrated by the finding that when raw soya flour is administered following a course of as little as 5 mg/kg azaserine weekly for only 3 weeks, pancreatic cancer develops in the treated rats. However, raw soya flour, like many other promoters, also seems capable of producing pancreatic carcinoma when administered alone for a sufficiently long period, although it is not possible to exclude the possibility that even in this case some unidentified environmental carcinogen has been promoted. Whatever the nature of the mechanism of raw soya flour-induced pancreatic carcinogenesis, the effects are exerted throughout the gland. As with other pancreatic carcinogens (*40*), foci of phenotypically altered cells develop in all parts of the pancreas. The mitotic activity of the cells in these foci is abnormally increased, suggesting that these cells are either more susceptible to the continuing hormonal drive provided by feeding raw soya flour, or less sensitive to normal inhibition of continuing pancreatic growth.

The lifespan of the neoplastically altered cells is probably shortened, as evidenced by the considerable degree of apoptosis within the foci. These foci, at all stages of development, must be considered neoplastic rather than "hyperplastic," and depend for further growth and frankly malignant change only on the presence of a promoter. Promotion is provided by dietary factors such as raw soya flour and probably fats. The raw soya flour acts especially powerfully if applied regularly but intermittently, rather than continuously. It seems that the drive to cellular replication within the neoplastic foci is greater with intermittent stimulation of growth, followed by rest, than by continuous exposure to the (hormonal) growth-producing stimulus. Perhaps in the latter situation (of continuous exposure to cholecystokinin) there is down-regulation of the cholecystokinin receptors, or desensitization (*41*) of the neoplastic cells, while cells exposed intermittently are not subject to receptor loss and do not lose functional (proliferative) capacity.

It is not yet clear how the association between ethionine-induced pancreatitis and the augmented incidence of pancreatic cancer is to be interpreted. Either the ethionine is acting as a genotoxic pancreatic carcinogen, which is promoted by raw soya flour, or the initiation provided by raw soya flour is promoted by the repair processes in the pancreas following ethionine pancreatitis.

With other models of experimental pancreatic cancer, there has been considerable discussion about the nomenclature and significance of the aggregates of phenotypically abnormal cells which occur and develop during the course of time (*40*). It seems to us that, in this model at least, there is no real distinction between different stages of "foci," "nodules" and "adenomata" regarding potential for development into frank carcinoma. "Reversibility" of the smaller aggregates of cells presumably merely reflects the tendency of these cells to become phenotypically (and functionally) more "normal" if the stimulus to growth (provided by a promoter or promoting situation) is removed. However, the cells have been "initiated" and therefore remain potentially precursors of pancreatic cancer if the growth-promoting circumstances recur.

Screening of Carcinogens

Potentiation of the effects of subcarcinogenic effects of azaserine provides a very sensitive model for testing or screening for other genotoxic carcinogens. Since, like azaserine, this type of pancreatic carcinogen must be given in very large doses in order to permit development of pancreatic cancer, it seems probable that the promotion provided by raw soya flour will permit detection of potential carcinogens in much smaller dosage. It is, of course, essential to quantitate the numbers of neoplastic foci within the pancreas in order to detect early potentiation (*35*). In longer term studies, it becomes necessary to differentiate between the effects of the potentiated carcinogen and the background proliferation evoked by the promoting raw soya flour. Under these circumstances, more elaborate morphometric techniques are required to determine the amount of pancreatic tissue replaced by abnormally proliferating masses of cells (*42,43*). In view of the likelihood that under normal conditions man is rarely exposed to very large amounts of carcinogens, we consider that screening for environmental pancreatic carinogens with sensitized (promoter-fed) animals is much more likely to reflect normal environmental circumstances and therefore to permit identification of chemical agents which have potential carcinogenic risk for human (as well as rat) pancreas. Earlier studies, using single chemicals without promotion (*44*) may therefore not have been sufficiently sensitive to detect possible genotoxic pancreatic carcinogens. A possible example is the failure to identify ethionine as a pancreatic carcinogen in non-soya chow-fed animals.

Just as the raw soya flour-fed rat has a pancreas which is admirably sensitive for testing genotoxic carcinogens, so the rat which has been given a subcarcinogenic dose of azaserine is suitable for screening epigenetic (promoting) carcinogens, since this type of carcinogen will act like raw soya flour in potentiating

the development of pancreatic cancer in appropriately treated rats.

Testing Anti-Cancer Drugs and Regimes

In view of the high yield of neoplastic foci, nodules and carcinomata in rats given azaserine and fed raw soya flour, therapeutic agents or dietary regimes with potential activity against pancreatic cancer can be tested for efficacy on all stages of pancreatic carcinogenesis. For example, using melphalan, we found no rats with pancreatic cancer in animals fed raw soya flour for more than 60 weeks (compared with an incidence of 12% in rats not receiving melphalan).

Role of Dietary Factors in Pancreatic Carcinogenesis in Rats

The promoting and weak carcinogenic effects of raw soya flour must be taken into account when testing chemicals for pancreatic carcinogenic effects. In this connection, some of the commercially available rat chows contained raw soya flour when originally tested (3 yr ago). It is also necessary to bear in mind that apparently "spontaneous" nodules may reflect the presence of carcinogens in the environment of the animals. For example, *N*-nitrosamines have been found in animal feeds and bedding (*45*).

Implications for Potential Human Pancreatic Cancer

Nothing is known about the effects of soybean products on the human pancreas, nor to what extent (if any) our model of experimental pancreatic carcinoma reflects human pancreatic carcinogenesis, either theoretically or in fact. The matter is clearly important, in view of the worldwide increase in the incidence of pancreatic cancer (*46*) and the potential significance of animal models for identifying environmental factors which are etiologically important in the genesis of human cancer (*47–49*).

Soybean derivatives, in various forms (*50–53*), are used widely in human nutrition. Soybean trypsin inhibitors can inhibit human trypsin and chymotrypsin (*54*), and it seems possible that man shows negative feedback control of pancreatic secretion (and, therefore, possibly pancreatic growth) like the rat (unpublished observations). If this is the case, it is important that all soya products for human consumption be tested for their effects on pancreatic growth in the rat. The effect of long-term feeding of textured soya proteins, used as meat expanders, on the rat pancreas has been studied by the manufacturers, but the results are not readily available. This, and related types of information, should be published.

Two population groups may be particularly at risk if soya bean products containing active protease inhibitors affect the human pancreas in a manner similar to the rat. One is the adult group of patients suffering from hyperlipoproteinaemia, in whom soybean protein-containing diets (in which soybean protein replaces all animal protein) are used in order to reduce blood levels of cholesterol. This type of patient could be exposed to a relatively high intake of trypsin inhibitor.

The other group comprises infants, who are often fed soya-containing products. In particular, cow milk-allergic infants may subsist on soya-derived milk for many months (*55*). The magnitude of the potential problem can be gauged from the estimate of 10,000 to 30,000 infants supposedly suffering from allergy to cows' milk in the USA per annum (*56*).

It has been stated (*57*) that over 85% of the original content of trypsin inhibitor of raw soya flour is destroyed during heat treatment of the soy protein used in milks. It has also been reported that when a soy protein preparation was administered to rats for 3 weeks, no histological changes were observed in the pancreas and pancreatic weights were similar to those resulting from administration of a diet in which casein was the source of protein.

We have studied the trypsin-inhibitory content of soy milks. Two points require emphasis. We have confirmed that casein has anti-tryptic properties (*58*) and that some, at least, of the trypsin-inhibitory effects can be found in cow milk-derived infant formulae (Fig. 2). Since casein elicits discharge of pancreatic enzymes when administered to rats (while albumin does not) (*59*) in a manner perhaps similar to raw soya flour, it seems probable that casein should not be used as "control" protein when studying the nutritional or carcinogenic effects of soybean protein. Indeed, it seems possible that cow milk may promote pancreatic carcinogenesis in rats given subthreshold amounts of an exogenous carcinogen (*60*).

We have found that soya milks (two preparations)

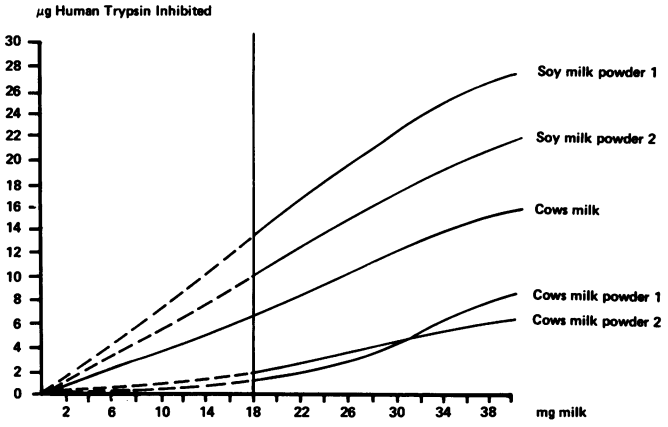

FIGURE 2. Inhibition of human trypsin by two preparations of soy milk and two preparations derived from cows' milk, as well as fresh cows' milk. Human breast milk did not show inhibitory activity. Method of assay as in (*65*). The dotted lines indicate extrapolated values below the sensitivity of the method.

contain trypsin-inhibitory activity which is not destroyed by heat and which is active against human trypsin (as found in duodenal aspirate after stimulation of the healthy pancreas with secretin and cholecystokinin) (unpublished observations) (Fig. 2). Heat may not destroy the protease inhibitors of soybean extracts (*61*) and, indeed, a finding similar to ours indicates that 'resterilisation' does not significantly reduce the residual soybean trypsin-inhibitory activity (*57*). Some of the heat resistance of the trypsin inhibitors may be attributable to the presence of the relatively heat-stable Bowman-Birk inhibitor, which is also relatively resistant to degradation by gastric juice (*62*). Control studies with human breast milk showed no trypsin-inhibitory activity. The trypsin-inhibitory content of the amount of soy milk powder recommended for one feed was sufficient to inhibit the tryptic activity of about 40 mg of (adult) human trypsin.

That soy milks may have effects on the pancreas of human infants can be inferred from a recent report which has shown that stimulation with cholecystokinin increases the enzymic activities of duodenal aspirate from infants fed soya milk, but not from infants fed cow milk (*63*). The latter results cannot be satisfactorily interpreted in terms of pancreatic function (since only enzymic activities, and not outputs, were measured) but if the reported results do reflect enzymic outputs, then the soy milks may indeed be acting in man as in the rat.

Unfortunately, we have not been able to obtain facilities for testing the effect of soy milks on the rat pancreas. Our results have been presented to the manufacturers of soy milks and other products and to appropriate national regulatory authorities. However, since the bearing (if any) which soy-related pancreatic carcinogenesis in the rat has on human disease is not known, no regulatory action can be specified at the present time. The general problems underlying governmental control of food safety have been reviewed previously (*64*). We would strongly urge that extensive feeding studies be undertaken in rats, not only of soy milks and other products, but also of these materials combined with subthreshold amounts of, e.g., azaserine, since it seems probable that if there is no effect on the rat pancreas, no noxious effect will be exerted on the human pancreas either.

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