Evolution of the Health Benefits of Soy Isoflavones (44249)

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Abstract. **Soy is a unique dietary source of the isoflavones, genistein and daidzein. It has been part of the Southeast Asian diet for nearly five millenia, whereas consumption of soy in the United States and Western Europe has been limited to the 20th century. Heavy consumption of soy in Southeast Asian populations is associated with reduction in the rates of certain cancers and cardiovascular disease. Recent experimental evidence suggests that phytochemicals in soy are responsible for its beneficial effects, which may also include prevention of osteoporosis, a hereditary chronic nose bleed syndrome, and autoimmune diseases. Exposure of soy formula–fed infants to the potential estrogenizing effects of the isoflavones is limited by the first pass effect of the liver following the uptake of isoflavones from the gut. Several mechanisms of action of isoflavones have been proposed—both through estrogen-dependent and estrogen-independent pathways.** [P.S.E.B.M. 1998, Vol 217]

The Importance of Polyphenolics to Plants

Polyphenolics, such as the bioflavonoids and the coumestans, are widely found in members of the plant kingdom (1). In many plants they are present in high concentrations, suggesting that they have an important role in the life of the plant. These functions include activities as phytoalexins by inhibiting the action of invading microorganisms (2) and as specific molecular signals to other microorganisms that take part in symbiotic relationships with the plant (3–7). *Bradyrhizobium sp.* that forms root nodules on leguminous plants, is attracted to the plant when specific flavonoids and isoflavonoids are secreted from the plant roots. For instance, when the isoflavonoid genistein (5,7,4′-trihydroxyisoflavone) (Fig. 1) is secreted from the roots of soybeans, it interacts with the nodulation genes of *Bradyrhizobium japonicum;* the microorganism in turn issues a set of specific lipo-chitin oligosaccharides (8, 9). These induce root hair deformation and curling, and the production of nodule primordia. The root nodules containing the bacterium convert atmospheric nitrogen to ammonia that can be used directly by the plant. This fortuitous symbiosis between leguminous plants and bacteria was appreciated by the ancient Romans, 2000 years ago, as an important part of crop rotation in agriculture. This property is widely exploited by American farmers today.

Bioflavonoids and Mammals

The flavonoids and isoflavonoids derived from edible plants represent an important class of xenobiotics that humans and other animals have been exposed to throughout their respective evolutions. The foraging animals have established an adaptive co-existence with these compounds. However, human exposure to the isoflavonoids has been more restricted due to their limited distribution among the edible plants, the tropical legumes being the principal source of the enzyme chalcone isomerase, which is necessary for isoflavonoid biosynthesis. Of the foods familiar to 20th century consumers, soybeans are the richest source of isoflavones, containing mg/g amounts of the isoflavones genistein and daidzein (7,4′-dihydroxyisoflavone) (10), mostly as esterified β-glucosides (11–13) (Fig. 2).

History of Soy as a Source of Foods

The first recorded use of soy is contained in the Materia Medica of the Chinese Emperor Shen Nung in 2838 BC (14). Tofu (a protein-rich curd made from a hot water extract of soybeans) was developed in China and was introduced into Japan and Korea by Buddhist missionaries in the period between the 2nd and 7th centuries. From the 13–19th

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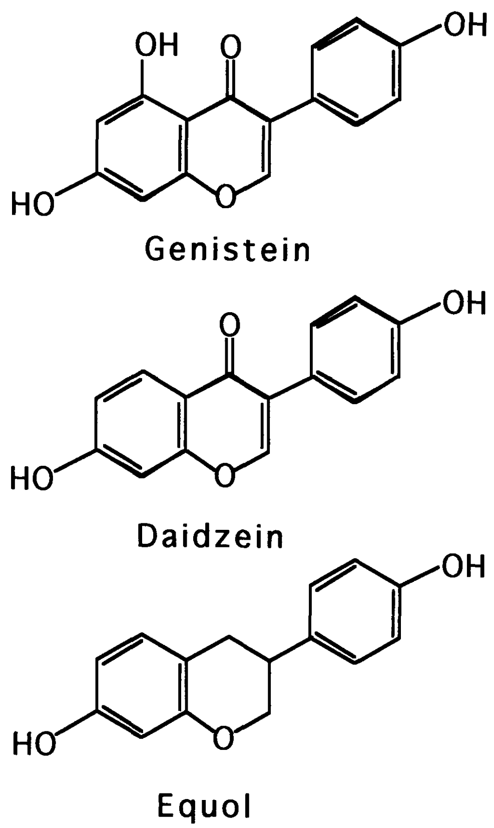

Figure 1. Chemical structure of the principal isoflavones in soy and of equol, a biologically active metabolite of daidzein.

centuries, tofu was a revered food of the courts of the Chinese Emperors (Ming to Chin dynasties) (15).

Peoples from Southeast Asia have used soybeans as a staple in their diet for several centuries—the protein from soy foods comprising 20%–60% of their daily protein intake. However, the first recorded growth of soybeans in the West did not occur until in the 18th century in botanical gardens in England and France.

The immigration of Chinese laborers in the second half of the 19th century led to the introduction of soy foods to the United States. In 1890, the growing of soybeans was begun in Agricultural Stations all over the country. By the turn of the 20th century, the USDA had sponsored studies to identify, out of the 10,000 known varieties, specific strains of soybeans that grew optimally under the different soil conditions and temperatures and in the length of days in each of the major farming states in the United States. Since then, the USA has become the world's largest producer of soybeans, with a large part of the crop being exported.

Soybeans are converted to a variety of food products. In Southeast Asia, besides soy milk and tofu, many of these foods arise from fermentation (e.g., miso, soy bean paste, tempeh) (Fig. 3). Americans mostly use soy for the production of vegetable oil, which is prepared by extracting crushed soybeans with hexane. The defatted soybeans after removal of residual solvent are pulverized to form soy flour, which contains approximately 50% by weight of protein

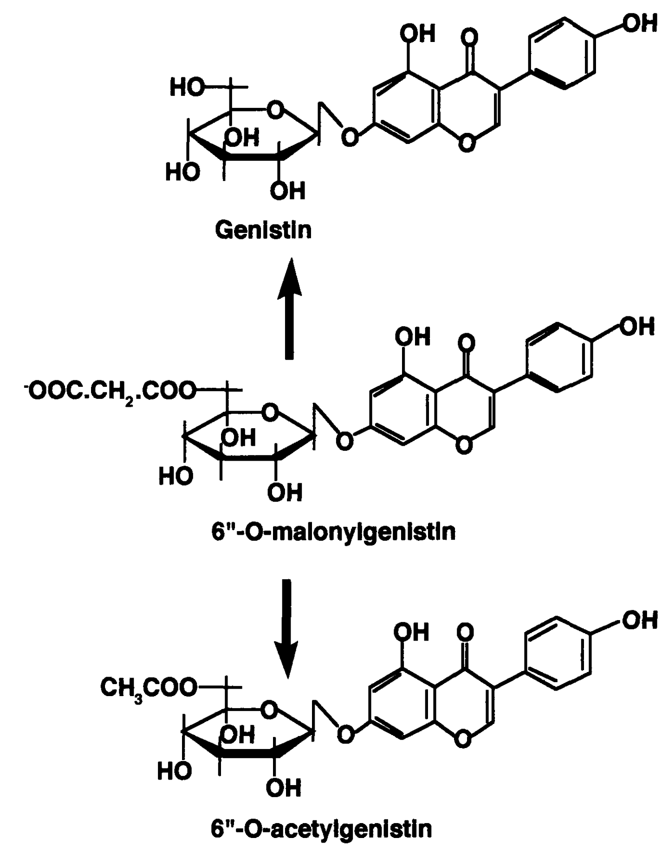

Figure 2. Chemical structures of the glycosides of genistein in soy. 6"-*O*-malonylgenistin is the principal glucoside in the soybean. It is converted to genistin by hydrolysis during the preparation of soy milk and tofu. The formation of 6"-*O*-acetylgenistin occurs during the dry heating processes used to prepare toasted soy flour and isolated soy protein.

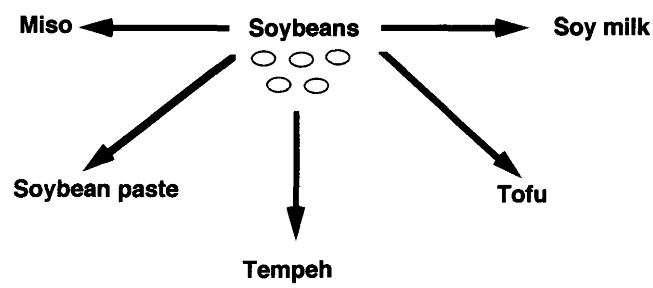

Figure 3. Soy foods in the Southeast Asian diet. Miso, soybean paste, and tempeh are prepared by fermenting soy beans and contain a large proportion of the isoflavones as their aglucones. Tofu and soy milk contain only the β-glucosides, genistin, and daidzin.

(Fig. 4). Soy flour comes in several grades based on the extent to which it is heat-treated. The least heat-treated grade is used in the preparation of protein-enriched breads. Heat treatment inactivates the protease inhibitors contained in soy. The majority (more than 95%) of soy flour is toasted and fed to livestock and other commercially bred animals.

Currently, tofu can be found in most grocery stores in the United States. Another soy food that has found a niche in the American culture is soy milk. It is used by the many people who have intestinal discomfort and diarrhea when drinking cow's milk, a problem created by their lactase deficiency.

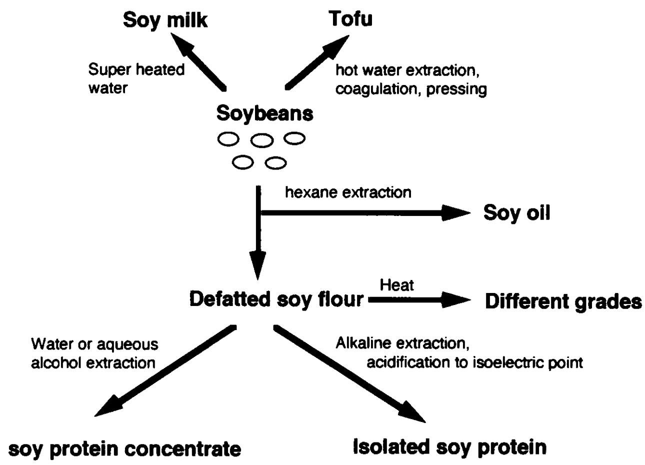

Figure 4. Soy food materials prepared for the US market. Tofu and soy milk are also available in "lite" forms containing approximately 1% fat. Aqueous alcohol extraction of soy flour removes >95% of the isoflavones. Variable amounts of the isoflavones (all conjugate forms) are present in isolated soy protein preparation.

Soy flour is further treated to generate products with a high protein content (Fig. 4). Aqueous washing of soy flour removes the soluble carbohydrates and increases the percentage of protein to 60%–70%. In order to get taste-free and color-free soy protein preparations for use by the food industry, some soy processors have washed soy flour with a mixture of water and alcohol. This procedure effectively removes almost all the phytochemicals in soy, a possible disadvantage from a health perspective for any food.

Isolated soy protein is prepared by first dissolving the proteins in soy flour (thereby leaving behind the complex, insoluble carbohydrates) and precipitating them at their isoelectric point (thereby leaving the soluble carbohydrates in solution) (Fig. 4). The dried product contains at least 90% protein.

The use of soy in All-American burger sandwiches during World War II and in the school lunch programs did not lead to a positive public perception of soy; however, recent improvements in food technology have enabled the soy burger to gain substantial credibility, particularly in the younger age groups and those opposed to eating meat. Soy burgers (frequently called ''gardenburgers'') can be found in the menus of several family-type restaurant chains (e.g., Ruby Tuesday's), and in favored restaurants even in the South, where barbecued meats often dominate. In London soy burgers are found alongside Big Macs and Quarter Pounders on the MacDonald's menu. The recent association in Great Britain of a new form of the neurodegenerative Creutzfeld-Jakob disease with consumption of beef products from cows infected with ''Mad Cow Disease'' can only hasten the establishment of the soy burger as a frontline fast food product.

Your Health and Soy

Ancient Chinese texts speak of the medicinal value of soy, albeit that they favored the use of the black soybean (kuroname) for medical purposes over the more familiar yellow bean grown in the United States. The first use of soy to give a health benefit in the US occurred in 1910 when it was recommended for use by diabetics (16). The rationale for its use was its low glycogen content. The discovery of insulin by Banting and Bess (17) quickly superseded this use of soy.

Many of the problems associated with the introduction of soy as a food in the United States arose because of the abundant amount of protease inhibitors in soy (18). Initial comparison of the nutritious value of soy protein with meat, eggs, and dairymilk proteins was hampered by the incomplete digestion of soy protein. This led to apparent nonequivalence of soy protein, an error that persisted for 60 years. It is now accepted that soy protein is every bit as good, gram for gram, as the traditional sources of protein (19). As a consequence, soy proteins are increasingly attractive to the food industry from the economic standpoint.

The health benefits of soy that have been deduced by epidemiological studies and by clinical and laboratory experiments may depend on the ''whole bean,'' or on the individual chemical components. In the case of the prevention of cancer, several agents that have anticancer properties have been identified in soy (20). Although the isoflavones are one of the most promising of these agents, the role of interactions between the components of the soy bean are largely unexplored.

Soy and Cardiovascular Diseases

In the 1970s investigations were begun on the effect of a shift in the diet from animal proteins to plant proteins on plasma cholesterol concentrations. Carroll *et al.* (21) were the first to report that soy protein lowered plasma cholesterol in hypercholesterolemic rabbits. This was quickly followed by studies in hypercholesterolemic men by Sitori's group in Italy (22). Many reports, which followed over the next 20 years, confirmed these findings to a greater or lesser extent (23). Nonetheless, it was not until publication of a meta-analysis by Anderson *et al.* (24) in 1995 that the scientific community was prepared to accept that soy was associated with a lowering of plasma cholesterol. In part, this has been due to a lack of appreciation of the role of the phytoestrogens, the isoflavones, in soy. The various sources of soy used in the earlier studies were not controlled for their isoflavone content despite studies published more than 20 years ago which suggested that biochanin A and formomonetin (the 4′-methyl ethers of the soy isoflavones genistein and daidzein) are hypolipidemic (25). In an accompanying article in this volume, Clarkson *et al.* (26) describe exciting data obtained in a monkey model of atherogenesis where they show that removal of the phytochemicals (including the isoflavones) from soy protein by aqueous alcohol extraction eliminates most of the beneficial effects observed with untreated soy. It appears now that a soy-based diet provides protection from the risk of death from cardiovascular disease and may explain the low incidence of this disease in China.

Soy and Cancer

From epidemiological studies, the consumption of soy is associated with a lowered risk of breast cancer, lung cancer, leukemia, and prostate cancer (27). Evidence in support of these associations has come from studies with cultured human breast cancer cells (28), leukemic cells (29), and prostate cancer cell lines (30), and from models of breast cancer (31–34). In an accompanying article in this volume, Lamartiniere *et al.* (35) conclude that in the case of breast cancer risk, the beneficial effects of soy and the isoflavones therein occur prior to and during puberty by accelerating the rate of differentiation of the epithelial cells of the breast (36). In contrast, in prostate cancer the beneficial effects of soy may occur during the promotional phase since young American and Asian men have the same incidence of latent cancerous lesions in the prostate (37, 38). Furthermore, Asian men who emigrated to the United States had an increasing risk of prostate cancer the earlier in their life they came to the US (39).

Beneficial Effects of Soy and Its Isoflavones on Menopausal Symptoms

As the life expectancy continues to rise, osteoporosis is becoming an increasingly important aspect of health care costs (40). Currently, the female baby boomers in the United States are dealing with menopause, a period associated with the onset of risk of osteoporosis. Estrogen replacement therapy started early enough can prevent this and other perimenopausal problems. However, the increased risk of endometrial cancer caused by estrogen therapy is of concern in these women (41) and alternative drugs are being investigated. One of these, Ipriflavone (7-isopropylisoflavone) (Fig. 5), is effective at a dose of 600 mg/day in preventing both postmenopausal (42) and senile osteoporosis (43). Interestingly, one of Ipriflavone's metabolites is daidzein (44)

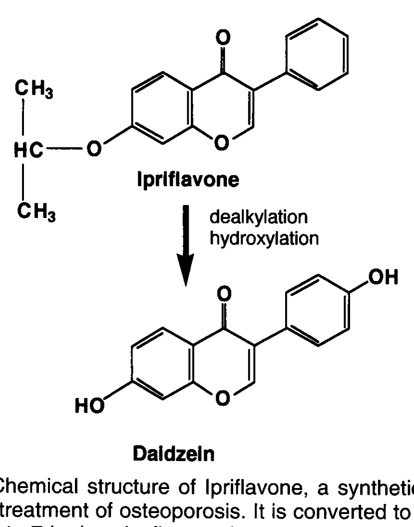

Figure 5. Chemical structure of Ipriflavone, a synthetic isoflavone used in the treatment of osteoporosis. It is converted to daidzein by dealkylation to 7-hydroxyisoflavone followed by 4′-hydroxylation.

(Fig. 5). In a recent study, Blair *et al.* (45) have shown genistein administered in the diet can significantly increase the mass of weight bearing bones compared to control in rats made surgically postmenopausal.

Given how soy and its isoflavones lower the plasma cholesterol concentration, prevent osteoporosis, and perhaps other postmenopausal symptoms, and do not increase cancer risk (rather decrease it), they are currently being carefully examined as alternatives to estrogen replacement therapy.

Soy Infant Milk Formula and Estrogenicity

Over the past 30 years soy protein has been used in the preparation of infant milk formulas. These were developed for infants that had digestion and diarrheal problems when fed cow's milk. Despite their successful use over this period of time, a controversy has recently arisen over the possible effects of the isoflavonoids from soy on the infants at a developmental stage of life (46). The concern raised by critics of this use of soy is centered on the properties of isoflavones as phytoestrogens. Genistein, daidzein, and the daidzein metabolite equol (7,4′-dihydroxylsoflavan) (Fig. 1) have been shown by several investigators to displace ^{3}H-labelled 17β-estradiol from estrogen receptors in cytosols from rabbit (47) and sheep (48) uteri and from MCF-7 cells (49), a human breast cancer cell line. Their apparent relative binding capacities to the estradiol receptor in these assays are approximately 1/100 to 1/1000th of that of 17β-estradiol or the synthetic estrogen, diethylstilbestrol (DES) (50).

However, uterotrophic effects of genistein in the diet have been difficult to demonstrate: in mice, oral genistein is 1×10^5 times less potent than DES (50). This has parallels to the effects of oral doses of physiological estrogens. Despite the equivalence of 17β-estradiol, DES, and chemically modified estrogens in *in vitro* binding assays, estradiol is a very weak oral estrogen *in vivo*. This is due to the extensive first pass metabolism of 17β-estradiol to inactive metabolites before it enters the peripheral blood stream and hence before it reaches its normal biological targets. Endogenous estradiol (produced by the ovaries), on the other hand, enters the peripheral blood circulation directly.

To overcome this metabolic inactivation of estradiol, pharmacologists have employed two strategies. In one, estradiol is administered transdermally. In this regimen, appropriate serum estradiol levels can be achieved by a 20-fold lower transdermal dose (0.1 mg/day) than an oral estradiol dose (2 mg/day) (51). In the other widely used approach, 17β-estradiol is modified at the 17α-position with an ethinyl group, thus producing the orally active contraceptive, ethinyl estradiol (EE). This synthetic estrogen is at least 200 times more active than 17β-estradiol when equivalent doses are given orally (52).

Thus, projecting the estrogenic effect of any orally ingested estrogen from *in vitro* binding assays can be wildly inaccurate. It is crucial that the extent of uptake and initial metabolism of the estrogen is known before evaluation of estrogenicity *in vivo* is made.

The route of administration of estradiol also qualitatively affects the actions it causes. 17β-estradiol (2 mg/day) administered orally to postmenopausal women stimulates HDL apoA-1 levels; in contrast, transdermal estradiol (0.1 mg twice weekly), which as noted earlier gave a similar systemic potency, had no significant effect on HDL apoA-1 levels (53). This difference is attributed to the much higher level of estradiol that reaches the liver (the site of HDL apoA-1 synthesis) when it is administered orally. This may prove to be analogous to genistein since it may be the factor in soy protein that is responsible for the cholesterol lowering effect of soy (25, 26).

The isoflavones in soy infant milk formula consist of mostly β-glucoside conjugates. Because of their polar nature these conjugates are not absorbed from the intestine (54). In adults they have to be first hydrolyzed to genistein by intestinal bacteria that have β-glucosidase activity. Even then, the malonate esters of the β-glucosides are not hydrolyzed by the flora in the small intestine, but instead must wait for contact with much larger population of microorganisms in the large bowel (55).

Cruz *et al.* (56) have reported that isoflavones are present in urine of young infants, but it is not known what proportion of the ingested isoflavones in the diet is actually absorbed. This question needs to addressed urgently even though working with young infants represents a particular challenge to the investigator. The isoflavones in young infant urine could be a result of the absorption of the small amount of unconjugated isoflavones in the soy infant formula, rather than from the β-glucosides fraction.

It also appears that the isoflavones are metabolized even before they enter the blood stream (57) due to the expression of UDPG-glucuronyltransferases (UDPGTs) in the intestinal wall. In adults consuming soy, the β-glucuronide conjugates are the major form of isoflavones in peripheral blood (58). UDPGTs for substrates such as bilirubin in infants are expressed in the liver within a few days after birth (59); however, the ontogeny of UDPGTs in the intestine in the early months of life is not known.

Mechanism(s) of Action of Genistein

The role of genistein as an estrogen is controversial. In the total absence of physiological estrogens, genistein in the nM concentration range has weak estrogenic effects and can stimulate cell growth (60, 61). However, at higher genistein concentrations, genistein inhibits the proliferation of MCF-7 and T47D human breast cancer cells induced by estradiol (62). Furthermore, genistein as an inhibitor growth factor stimulated growth independently of whether the cancer cells contain estrogen receptors (28, 62). The recent discovery of a new estrogen receptor (ER beta) will require considerable refinement of our concepts of estrogen action in target tissues (63).

In 1987, it was shown by Akiyama *et al.* (64) that genistein is also a potent protein tyrosine kinase inhibitor. Many of the peptide growth factor signal transduction pathways that were implicated in certain cancers involve the action of tyrosine kinases; therefore, a circulating tyrosine kinase inhibitor such as genistein may have beneficial effects in the treatment of cancer (65, 66). Studies in our laboratory have shown that genistein is an equally effective inhibitor of the proliferation of MCF-7 and T47D cells induced by epidermal growth factor or 17b-estradiol (62).

The cellular targets of genistein may extend far beyond its immediate effects on the tyrosine kinase activity of growth factor receptors. These potential targets (DNA topoisomerases, cell differentiation, cell cycle events, apoptosis, angiogenesis, and reactive oxygen species) have been reviewed by Peterson (67) and by Barnes *et al.* (68).

The antiangiogenic effect of genistein (69) may be important in the treatment of hereditary hemorrhagic talengiectasia (HHT), a severe familial disorder leading to abnormal blood vessel development in the nose, lung, and the GI tract (70). The genetic basis for this disease has been recently reported (71). The damaged gene encodes endoglin, an accessory protein involved in the function of transforming growth factor (TGF) β receptor (72). TGF-β is an inhibitor of cell cycle events. Disruption of its function appears to alter regulation of the control and growth of blood vessel architecture at the arteriovenous junction, leading to expansion of the post capillary venules and hence the collection of blood in this area (70). In a pilot study, Korzenik and Barnes (unpublished data) have found that in two out of five HHT subjects treated with 2 × 20 g/day isolated soy protein, nose bleeds completely stopped. In two patients there was no effect. It is possible that the latter patients had the second genetic lesion reported in HHT (73).

Another emerging area of interest is the effects of genistein on inflammatory diseases. In an accompanying article in this volume, Mannick *et al.* describe the inhibitory effects of genistein on the ileitis induced in rats by trinitrobenzene sulfonate (74). This result confirms observations made several thousand years ago in the Chinese medical literature (75). Since genistein has been shown to attenuate growth factor and cytokine-induced signal transduction events in the cells of the immune system, so it may also have a role in prevention or treatment of autoimmune diseases.

Summary

The exposure of humans to soy phytoestrogens goes back nearly five millenia. Today those nations whose people consume large amounts of soy in their diet report a much lower incidence of many of the chronic diseases that are targets of public health policy in the United States. Systematic studies in laboratory animals and in clinical trials have shown that either soy or the phytoestrogens in it inhibit these chronic diseases. Rather than being bad actors, the isoflavones in soy appear to be the "good guys."

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