Bovine Growth Hormone: Human Food Safety Evaluation

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Scientists in the Food and Drug Administration (FDA), after reviewing the scientific literature and evaluating studies conducted by pharmaceutical companies, have concluded that the use of recombinant bovine growth hormone (rbGH) in dairy cattle presents no increased health risk to consumers. Bovine GH is not biologically active in humans, and oral toxicity studies have demonstrated that rbGH is not orally active in rats, a species responsive to parenterally administered bGH. Recombinant bGH treatment produces an increase in the concentration of insulin-like growth factor–I (IGF-I) in cow's milk. However, oral toxicity studies have shown that bovine IGF-I lacks oral activity in rats. Additionally, the concentration of IGF-I in milk of rbGH-treated cows is within the normal physiological range found in human breast milk, and IGF-I is denatured under conditions used to process cow's milk for infant formula. On the basis of estimates of the amount of protein absorbed intact in humans and the concentration of IGF-I in cow's milk during rbGH treatment, biologically significant levels of intact IGF-I would not be absorbed.

GROWTH HORMONE (GH) IS A PROTEIN PRODUCED IN THE pituitary gland of all animals and is an important endocrine factor for normal growth and lactation in mammals. It was known as early as the 1930s that injection of dairy cows with bovine pituitary extracts increased milk yield, and this increase was eventually attributed to bovine growth hormone (bGH; also called bovine somatotropin or bST). The limited supply and the impurity of pituitary-derived bGH, however, precluded its commercial use on dairy farms. The advent of biotechnology in the 1980s has allowed the production of large quantities of pure bGH through recombinant DNA processes. Subsequently, several pharmaceutical firms have developed rbGH for administration to dairy cows to increase milk yield and the efficiency of milk production and are currently conducting studies necessary for evaluation of these products by the FDA.

Bovine GH treatment increases milk production by affecting several physiological processes (*1*). In general, there is an increased mammary uptake of nutrients used for milk synthesis accompanied by altered metabolism in other tissues, which results in the increased availability of these nutrients for milk synthesis. These changes in tissue metabolism initiated by bGH involve both direct effects and indirect effects mediated by insulin-like growth factors (IGFs).

Some consumers have become concerned about the use of rbGH in dairy cows as a result of reports from the news media of allegations of potential hazards. Although FDA scientists have determined that milk and meat from rbGH-treated animals are safe for human consumption (*2*), questions have remained in the mind of the consumer regarding the regulatory process within the FDA that permits marketing of food products from animals used in investigational studies and the scientific basis for decisions regarding the human safety of such products. The purpose of this article is to address these concerns by briefly explaining the approval process within the FDA and to summarize the scientific information used by the agency to evaluate the human safety of these products.

New Animal Drug Regulation

The FDA has the responsibility of enforcing the Federal Food, Drug, and Cosmetic Act (FD&C Act), and the enforcement authority for animal drugs is delegated to its Center for Veterinary Medicine (CVM). Before approving a new animal drug, the FDA requires that the pharmaceutical company demonstrate that food products from treated animals are safe for human consumption. In addition, the company must show that the drug is effective and safe for the animal, and that the manufacture of the drug will not adversely affect the environment. These general requirements are outlined in the *Code of Federal Regulations* (*3*). The efficacy and target animal safety studies must include trials in several different geographical locations in the United States under typical conditions of use. To conduct clinical studies with investigational drugs, the pharmaceutical companies must establish an Investigational New Animal Drug (INAD) application with the FDA, through which the agency controls the use of the unapproved compound in food animals. The label for the compound indicates that the drug is investigational and that animals treated with the drug must not be used for human food unless this use is expressly authorized by the FDA.

Under an INAD application, pharmaceutical companies may conduct the human food safety studies required for approval of their product. The results of these studies may be submitted to the CVM while the compound is still undergoing investigation. CVM scientists review the human food safety data and establish an appropriate period for drug withdrawal before slaughter, or a discard period for milk, which ensures that no unsafe residues are present in the food products. At that point, the FDA may authorize the use in human

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food of products from animals treated in investigational studies. Initially, investigators were required to discard milk while cows were being treated with rbGH and for 4 days or longer after the end of rbGH treatment and were not allowed to slaughter the cows for human consumption until 15 days after the last treatment. The pharmaceutical companies later completed the human food safety studies, and the results demonstrated that a withdrawal period was not required. Under conditions of the INAD regulation, the FDA then permitted milk and meat from rbGH-treated cows to be marketed with no withdrawal period. Because the FDA requires the pharmaceutical companies to submit all studies they conducted on their products, the agency continues to receive human food safety information even after the requirements have been met.

The FDA's current human food safety requirements for protein drugs such as rbGH are discussed below. Guidelines for conducting safety studies for nonprotein drugs will not be discussed here but can be obtained from the CVM (*4*).

Data quality assurance. The pharmaceutical companies provide descriptions of the human food safety studies and summaries of results, but ultimately it is the FDA that decides on the integrity of the data. The FDA has established specific guidelines ("Good Laboratory Practices") to ensure that the data obtained from the pharmaceutical companies provide accurate and reliable information (*5*). The companies also submit the raw data from all safety studies that will form the basis of approval of the product; the submission permits CVM scientists to confirm the accuracy of the results and conclusions. CVM scientists may also order data audits and inspections of specific studies to aid in evaluating the adequacy of the data.

Human food safety requirements for protein products. With the advent of recombinant DNA techniques to produce easily purified proteins in large quantities, the investigation of protein products for use in food animals increased dramatically. The chemical nature, biological activity, and potential for harmful residues are better understood for protein products than for new chemical entities that are generally developed for use in food animals. The scientific literature provides a good background for understanding the biological effects of these products, and knowledge about digestion of proteins in the human gastrointestinal tract provides information on their potential for harmful residues. The FDA's "Guideline for Toxicological Testing" (*4*) provides for alternatives to the general tests outlined, depending on the potential exposure of people to residues and the possible biological effects of the compound, and the CVM has determined that these alternatives are more appropriate for protein products.

A determination of the potential for oral activity of the protein drug in test animals is initially required. The design of oral toxicity studies is based on the known biological activity of the particular protein, and the studies are generally conducted for at least 2 weeks. Some protein compounds are effective when administered orally, and there is evidence that short-term tests are adequate to determine this activity. If the initial toxicity study demonstrates that the protein is indeed orally active, additional testing may be required. If the protein product is biologically active in humans and will be used in lactating dairy animals, the potential for residues in milk needs to be addressed. The information from the oral toxicity test is coupled with residue data, when required, to provide a solid foundation for assessing potential health risks.

In evaluating the human food safety of rbGH, the FDA took into consideration the general nature of protein digestion and absorption in adults and neonates, the effects and mechanism of action of growth hormone, its effects on other growth factors, and the potential for biological and oral activity in humans. These considerations are discussed below.

Human Food Safety Considerations

Protein digestion. Ingested rbGH would be expected to be degraded in the human gastrointestinal tract in the same manner as other proteins. In adults, protein digestion products generally enter the blood almost entirely as free amino acids. Peptides may enter cells if their molecular weight is less than about 250 (*6*), and the extent to which a peptide enters the blood intact also depends on the rate of absorption and rate of intracellular hydrolysis. In neonates, the activity of various digestive enzymes ranges from 10 to 100% of adult levels. However, neonates, and even preterm infants, have the complement of enzymes necessary to digest protein efficiently, although digestive capacity is limited (*7*).

Absorption of intact proteins. Initially, uptake of intact proteins was considered to be limited to neonates and the mechanism of uptake has been studied in several species (*8*). The transport of intact proteins across the intestinal wall in mature animals has not been extensively studied; however, there is evidence that proteins may be absorbed intact (*9, 10*). In humans, this evidence relies on the presence of circulating antibodies to food proteins; however, no adverse reactions have been observed in the majority of individuals in response to protein absorption (*11*).

Whether full-term human neonates absorb a substantially greater amount of intact protein than older children and adults is still equivocal. The gut of the newly born infant is impermeable to a large variety of antibodies administered in colostrum or milk (*12*); however, absorption of foreign proteins must take place to some extent, as evidenced by the appearance of specific antibodies against proteins (*13*). The time of closure of gut permeability to proteins (gut closure) in the newborn has not been determined, but may occur before birth (*14*) or as long as 3 months after birth (*14, 15*). Because the time of gut closure appears to be quite variable among species, studies performed in other animals cannot easily be extrapolated to humans (*8, 10, 16*).

The conflicting results of studies to determine the extent of intact protein absorption by human neonates demonstrate the complexity of the system being studied. A variety of factors are involved, including the type of protein being studied, gestational age of the neonate, and perhaps feeding regimen (*14, 17–19*). However, uptake of macromolecules into intestinal epithelial cells does not appear to be any more significant in the full-term neonate than in the adult. Estimates of the amount absorbed are on the order of 1:10,000 to 1:50,000 of the protein load given orally (*11*).

Most protein and polypeptide drugs will have minimal activity, at most, when administered orally. However, it would be inappropriate to assume that a compound does not have oral activity simply because it is a protein. For example, two polypeptide-releasing factors, synthetic thyrotropin-releasing factor (TRF; a tripeptide) and synthetic gonadotropin-releasing hormone (GnRH; a decapeptide) display some oral activity (*20*) because of their low molecular weights or their high specific activities, or both. The molecular weights of synthetic TRF and GnRH are approximately 330 and 1,100 daltons, respectively; in contrast, the respective molecular weights of bGH and IGF-I are approximately 22,000 and 7,800 daltons.

Growth hormone. The effects of GH can be considered at two levels: the effects on cell proliferation and protein synthesis and the effects on metabolic factors (*1, 21–26*). In vivo and in vitro studies have demonstrated that GH exerts direct effects on some processes and indirect effects, mediated by insulin-like growth factors, on other processes. In some tissues GH may first induce differentiation of precursor cells and then increase production of IGFs in the differentiated cells, resulting in a mitogenic effect (*22, 27*). The physiological effects of GH are manifested in (i) anabolic effects

(such as, nitrogen accretion in growing animals and milk synthesis in lactating animals), (ii) effects on electrolytes (phosphorus, sodium, potassium, and calcium), (iii) effects on carbohydrate metabolism, (iv) effects on lipid metabolism, and (v) growth of cartilage and bone.

Bovine Growth Hormone: Human Food Safety

The evaluation of the human food safety of bGH was based on several factors: bGH is biologically inactive in humans, rbGH is orally inactive, and rbGH and bGH are biologically indistinguishable.

Species specificity. On the basis of studies in the 1950s, it was concluded that, although the physiological effects of GH could be demonstrated in animals, pituitary GH preparations from animals were not effective in humans (*24, 28, 29*). GH derived from human cadavers is effective, but GH derived from bovine (*30*), ovine, whale (*31*), and porcine (*32*) pituitaries is ineffective in humans. Although bGH and human GH (hGH) both have 191 amino acids, the amino acid sequence differs by approximately 35% (*33*). A reflection of this difference is the demonstration that bGH does not compete with hGH for binding sites in membranes from human tissues, including liver, indicating that bGH does not bind to GH receptors in human tissues (*34*).

The finding that GH from nonprimate species is ineffective in humans led to the application of the term "species-specific." Although it is apparent from animal studies that this terminology is not technically correct (for example, bGH is effective in rats), the terminology has continued to be used with the understanding that it implies a difference in sensitivity as one goes up the phylogenetic tree, with humans and monkeys being unresponsive to GH from lower species.

Fragment activity. To obtain a more plentiful source of GH for human therapy, attempts were made to produce a growth factor from animal-derived GH that would be active in humans. Chymotrypsinized bGH produced no anabolic or metabolic effects in patients (*31*). Limited tryptic digests of bGH retained some of the activity of intact bGH when administered parenterally to hypophysectomized rats (*30, 35–37*), but there was a progressive loss of growth-promoting activity in the rat as the number of hydrolyzed peptide bonds increased and a substantial reduction in activity when more than three bonds were split (*38*). Recombined fragments have approximately 10% of the activity of bGH in rats (*37*). In patients, parenteral administration of tryptic digests of bGH produced some of the metabolic effects seen after administration of hGH. However, large doses were required, and variable and opposite effects were observed (*35, 39*).

Toxicity studies of bGH. On the basis of background information obtained from the scientific literature, studies were designed by the CVM to demonstrate further the human food safety of rbGH. Initially, each sponsoring company conducted an oral toxicity study with their particular rbGH product. The primary sequence of these products was either the same as or differed only slightly from pituitary-derived bGH, because of the recombinant DNA techniques used by each of the companies. Differences occur only at the NH_2-terminus end of the protein. American Cyanamid's rbGH product has three additional amino acids, Met-Asp-Gln. Eli Lilly & Company's (Elanco) product contains the following additional amino acids, Met-Phe-Pro-Leu-Asp-Asp-Asp-Asp-Lys. Monsanto Agricultural Company's product has a single amino acid substitution of Met for Ala on the NH_2-terminus end, and the Upjohn Company's product is identical to pituitary-derived bGH.

Upjohn conducted a 26-day oral toxicity study in which normal rats were treated with rbGH at 0, 0.5, 5.0, or 50.0 mg/kg of body weight per day by gastric intubation; a separate group was given rbGH at 50 μg/rat per day by subcutaneous injection (*40*). Monsanto conducted two studies: a 28-day study in which normal rats were treated with rbGH at 0, 0.06, 0.6, or 6.0 mg/kg per day by gavage (*41*) and a 90-day study in which normal rats were treated with rbGH at 0, 0.1, 0.5, 5.0, or 50.0 mg/kg per day by gavage and a separate group was treated with 1 mg/kg per day by subcutaneous injection (*42*). American Cyanamid conducted at 15-day study in which normal rats were treated with rbGH at 0, 0.1, 1.0, or 10.0 mg/kg per day by gavage (*43*). Elanco conducted a 14-day study in which normal rats were given rbGH at 0, 0.05, 0.5, or 5.0 mg/kg per day by gavage and a separate group was treated with 0.1 mg/kg per day by subcutaneous injection (*44*).

Each study met the FDA's minimum requirements of treating rats with up to 100 times or more of the dose administered daily to dairy cattle on the basis of milligrams per kilogram of body weight and administration for at least 14 days. Therefore, the high dose chosen for each study varied according to the company's proposed dosage for treatment of dairy cattle. Negative results were obtained with oral administration of rbGH in all studies, and only the details of the study conducted for the longest duration will be presented here. The parameters examined in each study were comparable.

In a 90-day oral toxicity study conducted by Monsanto, rats were treated with rbGH either by gavage or subcutaneous injection (*42*). Body weight and food consumption were determined weekly. In

Table 1. Body weight changes (in grams) of control rats and rbGH-treated rats (means ± SD). Charles River CD rats were treated for 90 days with rbGH either by gavage or by subcutaneous administration. Groups of 30 rats per sex each were treated with rbGH orally by gavage; one group was treated with rbGH by subcutaneous injection; and one group of animals served as untreated controls. From (*42*) with permission ©1989 Monsanto Agricultural Company.

Study day	Body weight change (g) for dosage of rbGH (mg/kg per day)					
		Subcutaneous	Oral			
	0	1.0	0.1	0.5	5	50
			Males			
8	58 ± 8.5	72* ± 20.4	61 ± 11.9	62 ± 13.5	62 ± 12.5	59 ± 7.6
29	170 ± 20.3	207* ± 34.5	174 ± 22.7	178 ± 29.6	181 ± 23.4	176 ± 21.7
50	239 ± 29.8	294* ± 44.8	240 ± 29.4	241 ± 40.8	239 ± 28.1	243 ± 31.8
85	324 ± 39.2	432* ± 60.3	327 ± 39.1	318 ± 53.0	325 ± 46.3	328 ± 43.0
			Females			
8	24 ± 8.7	33* ± 6.5	21 ± 7.3	25 ± 7.1	25 ± 6.6	25 ± 7.1
29	81 ± 12.0	101* ± 13.6	69† ± 13.1	80 ± 19.2	81 ± 13.5	83 ± 14.0
50	110 ± 16.6	150* ± 18.9	99† ± 14.5	116 ± 23.1	112 ± 17.8	114 ± 17.0
85	148 ± 24.4	217* ± 32.3	140 ± 19.6	152 ± 31.0	147 ± 22.0	152 ± 20.5

*Rank augmented *t* test (protected) significant at the 0.1% level. †Rank augmented *t* test (protected) significant at the 1% level.

Table 2. Absolute organ weights (in grams) in control rats and rbGH-treated rats (means ± SD). Charles River CD rats (n = 30 rats per sex) were treated for 90 days with rbGH either by gavage or by subcutaneous administrations, and one group of animals served as untreated controls. From (*42*) with permission ©1989 Monsanto Agricultural Company.

Organ	Absolute organ weight (g) for dosage of rbGH (mg/kg per day)					
		Subcutaneous	Oral			
	0	1.0	0.1	0.5	5.0	50.0
			Males			
Kidneys	3.677 ± 0.30	4.188* ± 0.06	3.178 ± 0.06	3.695 ± 0.09	3.540 ± 0.03	3.544 ± 0.05
Liver	16.549 ± 3.00	20.364* ± 2.22	15.614 ± 1.47	15.740 ± 1.39	15.993 ± 1.94	15.098† ± 2.21
Heart	1.726 ± 0.51	1.941* ± 0.15	1.645 ± 0.16	1.608 ± 0.15	1.618 ± 0.14	1.640 ± 0.17
Spleen	0.912 ± 0.11	1.274* ± 0.21	0.910 ± 0.11	1.051† ± 0.18	0.987 ± 0.16	1.002 ± 0.17
			Females			
Kidneys	2.067 ± 0.22	2.464* ± 0.20	2.040 ± 0.10	2.170 ± 0.20	2.102 ± 0.22	2.025 ± 0.14
Liver	8.637 ± 0.88	11.146* ± 1.43	8.302 ± 0.57	8.754 ± 1.00	8.446 ± 0.92	8.297 ± 0.84
Heart	1.041 ± 0.07	1.215* ± 0.11	1.061 ± 0.06	1.101 ± 0.09	1.034 ± 0.09	1.070 ± 0.20
Spleen	0.585 ± 0.13	0.855* ± 0.10	0.601 ± 0.09	0.663† ± 0.12	0.630 ± 0.10	0.608 ± 0.09

*Rank augmented t test (protected) significant at the 0.1% level. †Rank augmented t test (protected) significant at the 5% level.

addition, blood samples were collected for extensive clinical chemistry and hematology examinations, and urinalysis parameters were determined. Gross pathology and microscopic examination of tissues were conducted on all animals at the termination of the study (*45*).

There were no treatment-related deaths or clinical findings. A marked increase in body weight gain and feed consumption was observed from week 2 throughout the treatment phase for rats given subcutaneous injections; differences in mean body weights reached 16% in males and 20% in females by study week 13, compared to the negative control group. Body weights were unchanged after oral administration of rbGH (Table 1). An increase in absolute organ weights accompanied the change in body weight in rats treated with rbGH subcutaneously (Table 2). Heart, liver, kidney, and spleen weights increased in both sexes, and in addition, adrenal weight in males and thymus and ovary weights in females increased (*42*). In contrast, there were no biologically significant increases in organ weights for rats given rbGH orally. Absolute spleen weight increased for males and females given rbGH orally at 0.5 mg/kg per day; however, the increase was not dose-related and was most likely an incidental finding. In rats treated subcutaneously, ratios of organ weight to body weight were increased for spleen and adrenal and decreased for testes in male rats, and increased for heart and spleen and decreased for brain in the female rats. In contrast, increases in ratios of organ weight to body weight were sporadic in the rats administered rbGH orally and were not treatment-related.

No toxicologically significant changes were noted in the clinical chemistry, hematology, or urinalysis parameters determined in rats administered rbGH orally. Significant changes in clinical chemistry and hematology parameters occurred only in the group that received rbGH by subcutaneous injection (*42*).

Pharmacokinetics of rbGH. There were no statistically significant differences in the distribution half-lives, terminal distribution half-lives, total body clearances, and volumes of distribution between rMet-bGH and a recombinant, naturally occurring variant, rAla-Val-bGH, in lactating Holstein cows (*46*). These results indicate that the body does not treat rMet-bGH as a protein distinct from a naturally occurring bGH variant. Similar results have been obtained in another study in which two recombinant forms of hGH were found to have equivalent potency and pharmacokinetics in cynomolgus monkeys (*47*). One recombinant form had an amino acid sequence identical to that of the natural pituitary hormone and the other form had an additional NH_2-terminal methionine.

Residue studies of bGH. Residue studies are not normally required for protein products unless: (i) the protein is orally active and a safe concentration is required, (ii) no adequate biological end point can be determined for toxicological testing, or (iii) the product will be used in lactating food animals and has the potential for biological activity in humans. For rbGH, none of the three exceptions applies; therefore, residue testing is not required. Although rbGH residue studies are not significant for human food safety considerations, some studies have been conducted to determine if bGH concentrations are increased in the milk of rbGH-treated cows. The analytical methods used by the pharmaceutical companies to determine the amount of bGH in the milk were exclusively radioimmunoassay (RIA) procedures. Each company developed its own RIA procedure; none of these procedures could distinguish between the pituitary-derived bGH and rbGH product.

American Cyanamid conducted two studies (*48*). In the first study, milk from 22 control cows and 27 cows receiving daily injections of 37.5 mg of rbGH (approximately three times the proposed dose) was assayed for bGH. In the control group, 21 of the 22 cows had detectable levels (≥1.0 ng/ml) of bGH in their milk ranging from 1.1 to 1.7 ng/ml. Concentrations of bGH in the milk from the treated cows ranged from 1.1 to 2.1 ng/ml. The average bGH concentrations in the milk of control cows and rbGH-treated cows were 1.3 and 1.4 ng/ml, respectively. In the second study, similar results were obtained with 12 cows in which the bGH concentrations in the rbGH-treated cows were in the same range as in the untreated cows.

Groenewegen *et al.* (*49*) conducted a study with three untreated cows and three cows treated with 10.6 mg of rbGH per day (approximately the proposed dose) beginning at 28 days postpartum. When comparing the milk samples collected from both groups, they found that levels (mean ± SEM) of bGH in milk from rbGH-treated cows (4.2 ± 1.9 ng/ml) were not significantly different from those found in nontreated cows (3.3 ± 1.7 ng/ml) ($P > 0.05$).

Although these very limited studies suggest that milk concentrations of bGH do not increase significantly as a result of the treatment of dairy cows with rbGH at the proposed doses, the need to pursue more definitive studies has already been stated as unnecessary because bGH is biologically inactive in humans and orally inactive. Additionally, it has also been determined that at least 90% of bGH activity is destroyed upon pasteurization of milk (*47*). Therefore, bGH residues do not present a human food safety concern.

Effects of rbGH treatment of cows on milk composition. The effects of rbGH treatment on the major components of milk, when present, are minor and primarily occur early in the treatment period before the cow's intake of dry matter is adjusted. Milk composition of

treated cows is well within the normal variation observed during the course of a lactation. Changes in milk fat and protein composition depend on the cow's energy and nitrogen balances, respectively, and generally are temporary effects. The principal carbohydrate in milk, lactose, is not altered by rbGH treatment, and there are no consistent changes in the milk content of calcium, phosphorus and other minerals, or several vitamins (*1, 50*). Thus, rbGH treatment appears to have no significant impact on the nutritional quality of milk.

Insulin-Like Growth Factors (IGFs)

Because it is known that IGFs mediate many of the effects of GH and concentrations of IGFs are regulated by GH (*51–53*), the FDA considered it important to determine the potential impact of IGFs on the human food safety of rbGH. Two main types of IGFs have been defined by their structural and immunological properties and receptor activity (*52*): IGF-I, a 70–amino acid polypeptide, which is identical to somatomedin-C (*54*), and IGF-II, a 67–amino acid polypeptide. IGF-I was chosen as the sole representative of growth factors influenced by GH, because it is the major factor mediating the effects of GH and is more potent than IGF-II. Several reviews have been published on the biological actions of IGFs (*23, 53, 55, 56*).

Because production of IGFs was initially thought to be primarily in the liver, IGFs were believed to act solely by an endocrine mechanism, producing their effects at a site distant from its production. However, a study by D'Ercole *et al.* (*57*) demonstrated that changes in tissue concentrations consistently preceded changes in serum IGF-I after injection of GH, and on this basis it was postulated that IGF-I may also exert its biological effects by an autocrine or paracrine mechanism. Later work confirmed that local production of IGFs appears to be important for producing cellular effects (*58*).

The IGFs have acute metabolic and long-term, growth-promoting effects. In vivo, bolus injections of IGF-I and IGF-II cause insulin-like effects on glucose homeostasis and metabolism, but have no effect on lipid synthesis (*59*). The fact that IGF-I exerts its long-term growth-promoting effect only when it is administered by subcutaneous infusion, but not when it is administered daily by intravenous or subcutaneous injection (*60*), reinforces the theory that IGFs act as local growth factors rather than as circulating mediators of GH effects. Studies in rats demonstrated that infusion of IGF-I causes a dose-dependent increase in body weight, tibial epiphyseal width, and thymidine incorporating activity. However, IGF-II has no effect on body weight and is three times less potent than IGF-I when the other two growth parameters are examined (*55, 61*).

Serum IGF-I levels in normal humans are lowest in umbilical cord blood (0.33 U/ml) and increase during the first 2 to 4 years (0.4 to 0.85 U/ml) (*51, 62*). Serum levels of IGF-I in adults are in the range of 1.1 to 1.5 U/ml (*51*) or 200 ng/ml (*52*), and plasma levels of IGF-II of approximately 650 ng/ml have been reported in adults. The plasma levels of IGF-I are highest in 12-year-old girls and 14-year-old boys, with concentrations reaching two- to threefold those in adults (*52*). The age-dependent pattern for IGF-II concentrations appears to be different from the pattern for IGF-I. Levels at birth are low but reach almost the normal adult levels in the 1-year-old child (*62*).

Human milk concentrations of IGF-I were measured during the first 9 days postpartum (*63*). The mean IGF-I concentration was 17.6 ng/ml at 1 day postpartum, 12.8 ng/ml at 2 days postpartum, and 6.8 ng/ml at 3 days postpartum. After 3 days postpartum, the IGF-I concentration stabilized over the following week at 7 to 8 ng/ml. In a later study (*64*), IGF-I concentrations in human milk were measured and ranged between 13 and 40 ng/ml at 6 to 8 weeks postpartum with a mean of 19 ng/ml.

Insulin-Like Growth Factor–I: Human Food Safety

Although a variety of growth factors may have specific effects on cells and cellular metabolism, IGF-I is the main factor known to be regulated by GH. Human and bovine IGF-I are identical (*65*), but treating dairy cattle with rbGH was not expected to cause an increase in IGF-I concentrations of biological significance to humans. This perception was based on the mechanism of action of IGF-I, the concentration of IGF-I found in human milk, preliminary information on the concentration of IGF-I in milk of rbGH-treated cows, the way in which milk is processed for infant formula, and our knowledge of protein absorption and digestion in adults and neonates. However, because of the general lack of information in the scientific literature regarding the oral activity of IGF-I, the CVM decided to obtain more information.

Table 3. Absolute organ weights [in grams except heart and spleen (in milligrams)] in hypophysectomized rats treated with rIGF-I (means ± SEM). Six groups of rats, approximately 6 to 7 weeks of age, were treated with rIGF-I for 2 weeks according to the following regimen: One group was given a saline control; another was given BSA (bovine serum albumin) as a negative "oral protein" control; additional groups were given rIGF-I by gavage and another group was given rIFG-I via a subcutaneously (s.c.) implanted osmotic minipump as a positive control. All groups contained 20 rats per sex except for the subcutaneously implanted group, which contained 10 rats per sex. Rats were treated for either 17 days by gavage or 15 days by continuous subcutaneous infusion. From (*66*) with permission ©1989 Eli Lilly & Company.

Organ	Absolute organ weight for dosage of BSA or rIGF-I (mg/kg per day)					
		Oral BSA	Oral rIGF-I			S.c. infusion rIGF-I
	0	1.0	0.01	0.1	1.0	1.0
			Males			
Kidneys	0.564 ± 0.01	0.556 ± 0.01	0.593 ± 0.01	0.583 ± 0.01	0.575 ± 0.01	0.720 ± 0.02*
Liver	2.925 ± 0.08	2.993 ± 0.08	3.048 ± 0.06	3.051 ± 0.10	2.867 ± 0.10	3.085 ± 0.19
Heart	279.6 ± 6.3	287.0 ± 5.9	280.2 ± 4.4	290.0 ± 4.6	267.6 ± 6.3	303.0 ± 14.4
Spleen	147.6 ± 8.8	151.3 ± 8.6	147.2 ± 5.7	149.6 ± 5.8	147.1 ± 6.6	239.6 ± 17.9*
			Females			
Kidneys	0.545 ± 0.01	0.567 ± 0.01	0.558 ± 0.01	0.560 ± 0.01	0.538 ± 0.01	0.716 ± 0.01*
Liver	2.742 ± 0.07	2.795 ± 0.09	2.747 ± 0.06	2.790 ± 0.08	2.571 ± 0.07	3.069 ± 0.12*
Heart	274.3 ± 7.1	272.3 ± 6.7	263.3 ± 7.2	270.8 ± 7.1	261.8 ± 7.7	302.3 ± 9.2
Spleen	132.2 ± 3.6	137.1 ± 4.0	127.5 ± 5.2	134.4 ± 2.6	137.2 ± 5.1	231.6 ± 5.3*

*Significantly different from control ($P < 0.05$); Dunnett's two-tailed t test.

Table 4. Average daily body weight gains (grams) for rats treated with IGF-I (least square means ± SEM). Male and female rats, approximately 36 days old, were treated with rIGF-I for 2 weeks according to the following regimen: two groups served as negative control groups for the gavage and subcutaneous (s.c.) routes of administration. Rats were treated with IGF-I by oral gavage or as positive control groups with administration of IGF-I by subcutaneous infusion (osmotic pump). The last group was treated with alanyl porcine GH by subcutaneous infusion and was a positive control group for growth effects with a known growth promotant. All groups contained 20 rats per sex and were treated for a period of at least 2 weeks. From (*67*) with permission ©1989 Monsanto Agricultural Company.

Body weight gain (g) for a dosage of rIGF-I or pGH							
Oral rIGF-I (mg/kg per day)				S.c. infusion rIGF-I (mg/rat per day)			S.c. infusion pGH (mg/rat per day)
0	0.02	0.2	2.0	0	0.05	0.2	4.0
				Males			
7.29 ± 0.137	8.03 ± 0.137	7.81 ± 0.137	8.34 ± 0.137*	7.61 ± 0.137	8.18 ± 0.137†	8.68 ± 0.137†	10.08 ± 0.137†
				Females			
4.04 ± 0.138	3.71 ± 0.138	3.92 ± 0.138	4.13 ± 0.138	4.09 ± 0.138	3.96 ± 0.138	4.83 ± 0.138†	8.37 ± 0.138†

*Significantly different from control ($P \leq 0.05$). †Significantly different from control ($P \leq 0.01$); Dunnett's two-tailed t test.

Toxicity studies of IGF-I. Elanco (*66*) and Monsanto (*67*) have conducted toxicity studies to determine whether IGF-I is active when administered orally. Both IGF-I oral toxicity studies are described in detail because they were conducted in different models, namely, hypophysectomized and normal rats. The IGF-I administered to the rats in both studies was a recombinant product with an identical sequence to the natural IGF-I.

Elanco conducted a 2-week oral toxicity study with rIGF-I in hypophysectomized rats (*66*). Rats were treated with rIGF-I at 0.01, 0.1, or 1.0 mg/kg per day by gavage (LD, MD, and HD, respectively) or at 1.0 mg/kg per day by subcutaneous infusion (s.c. group). There were also two negative control groups; one given saline and the other given bovine serum albumin (BSA) by gavage.

There were no treatment-related deaths or clinical signs. Mean body weight and mean body weight gain for the s.c. group were significantly higher than those for the negative controls, starting on day 3 and continuing throughout the study. At termination, body weights of the males and females in the s.c. group were 15 and 12% greater than those of controls, respectively. Body weight gain of female rats in the LD oral group was significantly lower than that for controls. The mean body weights and body weight gains in all other groups were not statistically different from those of the control group ($P > 0.05$) (*66*).

No treatment-related changes in the hematological parameters were observed in any of the groups. A moderate increase (approximately twofold) in absolute neutrophil values was seen in the s.c. group animals which may reflect the mild irritation associated with the subcutaneous minipump implant. Statistically significant changes in clinical chemistry parameters were generally limited to rats in the s.c. group and included decreases in blood urea nitrogen (BUN), creatinine, albumin, total protein, and globulin and increases in inorganic phosphorus and potassium. The only difference noted in rats treated by oral administration of rIGF-I was a biologically insignificant decrease in total protein in the HD males.

The only statistically significant differences in organ weights compared to controls were found in the s.c. group and included increased kidney, spleen, adrenal, and brain weights in males, and kidney, liver, and spleen weights in females (Table 3) (*45, 66*). Increases in relative organ weights included kidney weight in LD females, and kidney, spleen, and brain weights in the males and females of the s.c. group. Relative thyroid and parathyroid weight was decreased in MD males. None of the organ weight changes were accompanied by gross or microscopic changes. There were no compound-related changes in organ weights of animals treated with rIGF-I by gavage.

The results of this study (*66*) demonstrated that subcutaneous infusion of rIGF-I in hypophysectomized rats caused increased body weight; increased neutrophil count; decreased BUN, creatinine, and albumin; and increased relative kidney and spleen weights in both males and females. These changes are attributed to the physiologic effects of IGF-I. In contrast, oral treatment with rIGF-I at doses up to 1 mg/kg per day caused none of the changes seen in the rats treated subcutaneously.

A 2-week oral toxicity study with normal rats was conducted for Monsanto Agricultural Company by Hazleton Laboratories (*67*). Rats were treated with rIGF-I at 0.02, 0.2, or 2.0 mg/kg per day by gavage (LD, MD, and HD, respectively), or at 0.05 or 0.2 mg/rat per day by subcutaneous infusion (LD and HD s.c. groups, respectively). A negative control was included for each route of administration, and one group was treated with alanyl porcine GH as a positive control (pGH-treated group). Treatment was initiated on two consecutive days to accommodate the large number of rats to be implanted with osmotic pumps. The study was planned in blocks of rats so that all treatments were equally represented on each start date. Body weights were recorded twice weekly, and food consumption was recorded weekly.

All rats survived until the termination of the study, and no compound-related clinical signs were seen. A significant increase in body weight was seen throughout the study in males of the LD s.c. group and in both sexes of the HD s.c. and pGH-treated groups (Table 4). These findings were considered to be treatment-related.

The mean body weight for males in the HD oral group was slightly but significantly increased from day 7 of the study; average daily gain was also significantly increased. There was no significant increase in average daily gain in any of the males in the other gavage groups or in any of the females. When examined by block, it appeared that there was an increase in average daily gain only in the male rats of the HD oral group of block 2 (8.41 g/day versus 7.74 g/day for controls) and not in block 1 (8.27 g/day versus 8.10 g/day for controls). It is therefore questionable whether the overall increase in body weight in males of the HD oral group can be attributed to treatment with rIGF-I.

Significant changes in hematology, clinical chemistry, and urinalysis parameters were noted in both sexes of pGH-treated group (*67*). There was a slight but significant decrease in erythrocyte count, hemoglobin, and hematocrit, and a significantly increased platelet count in the females. Evaluation of the clinical chemistry data for this group revealed significantly increased total serum protein, serum albumin, albumin/globulin ratio, calcium, and total bilirubin, and significantly decreased chloride in both sexes. Males also showed a significant increase in creatinine and decrease in inorganic phosphorus and sodium. Females showed a significant decrease in aspartate transaminase. Urinalysis revealed a significant increase in urine osmolality for both sexes.

In contrast to the rats in the pGH-treated group, rats receiving rIGF-I via osmotic minipump showed minimal changes and those only at the high dose. Platelet count and BUN decreased significantly in both sexes, and creatinine decreased significantly in females. The only significant change noted in rats treated with rIGF-I by gavage was a slight decrease in hemoglobin for the females in the MD oral group without concomitant changes in erythrocyte count or hematocrit. No significant changes were seen in the HD oral group rats. The decrease in hemoglobin is not considered to be treatment-related.

Gross pathology revealed no notable differences between control and treated groups. In the pGH-treated group, significant increases were observed in adrenal (*67*), heart, spleen, kidney, and liver weights in both sexes (Table 5), and in brain (with brainstem) and ovary weights in females (*67*). In the HD s.c. group, significant increases were observed in kidney and heart weights of males and females and in adrenal and brain with brainstem weights in females; liver weight increased and testes weights decreased in males. The only organ weight changes noted in LD s.c. group were increases in kidney and liver weights in males. Liver weights of the HD oral group males were increased. No other statistically significant organ weight changes were noted for the other animals treated with rIGF-I by gavage ($P > 0.05$).

Changes noted in relative organ weights (*67*) are as follows: the pGH-treated group showed an increase in heart and liver weights and a decrease in relative brain weight for both sexes; an increase in adrenal and kidney weights and a decrease in relative testicular weight in males; and an increase in relative spleen weight and a decrease in relative ovary weight in females. The HD s.c. group showed an increase in kidney weight and a decrease in brain weight for both sexes, a decrease in relative testicular weight in males and an increase in relative spleen weight in females. The LD s.c. group showed only a decrease in relative brain weight in males. The only organ weight change noted in the rats treated with rIGF-I by gavage was an increase in the relative heart weight for males in the LD oral group.

Epiphyseal widths were increased in females of the HD s.c. group and both sexes of the pGH-treated group. Tibia lengths were increased in the LD s.c. group males and both sexes of the pGH-treated group. In the groups treated with rIGF-I by gavage, epiphyseal widths were decreased in both sexes of the HD group and tibia lengths were increased in the LD and HD group males. These findings (*67*) in the oral groups are considered contradictory in terms of effects of IGFs on growth indices and are therefore considered to be sporadic results.

The results of this study (*67*) demonstrate that subcutaneous infusion of rIGF-I in rats produces effects similar to those seen with subcutaneously injected GH. When administered orally, rIGF-I had no effect. Body weights of male rats given the high dose of rIGF-I by oral gavage showed a statistically significant increase. However, this increase was considered incidental because it occurred in only half of the male rats, the body weight of the female rats in the HD gavage group did not increase, serum levels of IGF-I were not increased in the HD animals as they were in the positive control groups, and there were no changes in hematology, clinical chemistry and urinalysis parameters, or organ weights that were consistent with the effects of GH or IGF-I, as observed in the positive control groups. Therefore, it was concluded that rIGF-I is orally inactive at doses up to 2 mg/kg per day.

Residue studies of IGF-I. Several companies conducted studies to determine the concentration of IGF-I in the milk of rbGH-treated and untreated cows. The analytical methods used by the companies are exclusively RIA procedures that putatively measure free IGF-I plus IGF-I bound to carrier proteins. Bound IGF-I is liberated by an acid-ethanol extraction step. Each company developed its own RIA and submitted the procedure to FDA for evaluation.

The survey of 100 raw bulk tank milk samples from a commercial processing plant was conducted to provide data on the naturally occurring range of IGF-I concentrations in salable milk (*68*). The mean IGF-I concentration (± SD) in these samples was 4.32 ± 1.09 ng/ml with a range of 1.27 to 8.10 ng/ml (Fig. 1).

The range of IGF-I concentrations was also determined in salable milk from 408 untreated cows from five Missouri dairy herds (*69*). The highest mean concentration of IGF-I in milk was detected in early lactation (days 6 to 15 postpartum, 6.2 ng/ml), after which

Table 5. Least square mean absolute organ weights (grams), tibia lengths (millimeters), and epiphyseal widths (millimeters) in control rats and rats treated with rIGF-I (± SEM). Male and female rats, approximately 36 days old, were treated with rIGF-I for 2 weeks according to the following regimen: two groups served as negative control groups for the gavage and subcutaneous routes of administration. Two groups of rats served as positive control groups with administration of IGF-I by subcutaneous infusion (osmotic pump). The last group was treated with alanyl porcine GH (pGH) by subcutaneous infusion and was a positive control group for growth effects with a known growth promotant. All groups contained 20 rats per sex and were treated for a period of at least 2 weeks. From (*67*) with permission ©1989 Monsanto Agricultural Company.

Organ	Growth measurements for a dosage of rIGF-I or pGH							
	Oral rIGF-I (mg/kg per day)				S.c. infusion rIGF-I (mg/rat per day)			S.c. infusion pGH (mg/rat per day)
	0	0.02	0.2	2.0	0	0.05	0.2	4.0
				Males				
Heart	1.01 ± 0.02	1.06 ± 0.02	1.01 ± 0.02	1.03 ± 0.02	0.99 ± 0.02	1.00 ± 0.02	1.05 ± 0.02*	1.28 ± 0.02†
Spleen	0.59 ± 0.02	0.60 ± 0.02	0.59 ± 0.02	0.64 ± 0.02	0.62 ± 0.02	0.63 ± 0.02	0.69 ± 0.02	0.70 ± 0.02*
Kidney	2.40 ± 0.05	2.41 ± 0.05	2.31 ± 0.05	2.39 ± 0.05	2.21 ± 0.04	2.35 ± 0.04*	2.45 ± 0.04†	2.63 ± 0.04†
Liver	8.07 ± 0.14	8.46 ± 0.14	8.12 ± 0.14	8.59 ± 0.14*	7.62 ± 0.14	8.10 ± 0.14*	8.19 ± 0.14†	11.98 ± 0.14†
Epiphyseal width	0.42 ± 0.01			0.38 ± 0.01†	0.41 ± 0.01		0.41 ± 0.01	0.45 ± 0.01†
Tibia length	36.1 ± 0.19	36.9 ± 0.19†	36.51 ± 0.19	36.9 ± 0.19†	35.7 ± 0.17	36.5 ± 0.17†	35.9 ± 0.17	36.3 ± 0.17†
				Females				
Heart	0.72 ± 0.02	0.71 ± 0.02	0.71 ± 0.02	0.73 ± 0.02	0.70 ± 0.02	0.70 ± 0.02	0.77 ± 0.02†	0.99 ± 0.02†
Spleen	0.40 ± 0.01	0.43 ± 0.01	0.40 ± 0.01	0.42 ± 0.01	0.42 ± 0.02	0.43 ± 0.02	0.53 ± 0.02†	0.62 ± 0.02†
Kidney	1.66 ± 0.03	1.53 ± 0.03	1.61 ± 0.03	1.62 ± 0.03	1.65 ± 0.04	1.60 ± 0.04	1.84 ± 0.04†	2.24 ± 0.04†
Liver	5.74 ± 0.12	5.64 ± 0.12	5.52 ± 0.12	5.78 ± 0.12	5.60 ± 0.13	5.57 ± 0.13	5.96 ± 0.13†	10.16 ± 0.13†
Epiphyseal width	0.32 ± 0.01			0.27 ± 0.01†	0.30 ± 0.01		0.33 ± 0.01*	0.41 ± 0.01†
Tibia length	34.0 ± 0.17	33.8 ± 0.17	33.7 ± 0.17	33.8 ± 0.17	33.4 ± 0.16	33.4 ± 0.16	33.6 ± 0.16	34.8 ± 0.16†

*Significantly different from control ($P \leq 0.05$). †Significantly different from control ($P \leq 0.01$); Dunnett's two-tailed t test. Epiphyseal widths were not measured in groups where data are not presented.

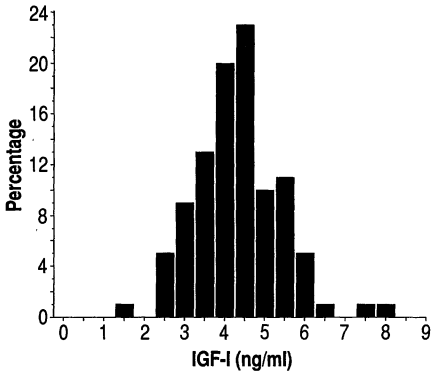

Fig. 1. The distribution of IGF-I concentrations in untreated cows in a survey of 100 raw bulk tank milk samples collected from a commercial processing plant.

milk concentrations declined. Multiparous animals had significantly higher mean milk IGF-I concentrations (2.83 ng/ml) than primiparous (first lactation) animals (2.15 ng/ml). Stage of lactation effects were detected in both parities, and the effect of parity was apparent at all stages of lactation. The survey studies determined that the concentration of IGF-I in milk of untreated cows is quite variable, ranging from <0.7 to 8.2 ng/ml in 95% of the cows with a maximum of 30.5 ng/ml, depending on parity and stage of lactation of the cow.

Schams and Karg (*70*) investigated the increase in IGF-I concentrations in the milk of cows treated with rbGH. In the first experiment, eight cows (four controls and four treated) of different breeds were injected subcutaneously with 640 mg of rbGH in a prolonged release formulation every 28 days (approximately the proposed dose). Milk samples were collected in the morning before the third injection and after on days 1, 3, 6, 8, 10, 13, 15, 17, 20, 22, 24, and 27 and after the fourth injection of rbGH on days 1, 3, 6, 8, 10, and 13. Mean amounts of IGF-I in the milk of treated cows were always higher than those found in the controls. The average IGF-I milk concentration found in the control cows was 28.4 ng/ml, and the average IGF-I milk concentration in the rbGH-treated cows was 35.5 ng/ml, representing an increase of 25% of the mean.

In another study conducted for Elanco (*71*), 36 cows that had completed at least one full lactation were given a single subcutaneous injection of 0, 320, or 640 mg of rbGH (12 cows per group). The concentration (mean ± SEM) of IGF-I in milk was significantly higher by day 3 in cows treated with 320 mg of rbGH (13.9 ± 1.35 ng/ml) than in the control cows (9.5 ± 1.35 ng/ml) ($P < 0.05$, protected t test), but not in those cows treated with 640 mg of rbGH (12.6 ± 1.41 ng/ml) ($P > 0.05$, protected t test). The values at 10, 17, and 24 days after treatment were also not significantly different for any of the groups ($P > 0.05$, protected t test).

White *et al.* (*72*) conducted a study to provide additional data about the effect of exogenous administration of rbGH on concentrations of IGF-I in milk. Eighteen lactating cows were administered subcutaneous injections of 500 mg of rbGH in a prolonged release formulation (approximately the proposed dose) or a sham injection at 14-day intervals (9 cows per group). IGF-I concentrations in milk were significantly increased in rbGH-treated cows, although the increases were numerically small and occurred only in injection cycles 2 and 3 of treatment (Table 6). The overall range of concentrations was similar for both groups: 2.16 to 9.04 ng/ml for the control group and 1.56 to 8.83 ng/ml for the rbGH treatment group.

Miller *et al.* (*73*) assessed the potential carryover of IGF-I in processed milk. IGF-I concentrations were measured in raw and pasteurized milk and in milk subjected to conditions similar to those used in the preparation of infant formula. Daily milk samples were obtained before and after pasteurization from a local commercial processing plant. The milk was pasteurized by standard procedures. Conditions used to process milk for infant formula (heating in a retort at 250°F for 15 min) can be simulated in the laboratory. Raw (unpasteurized) and pasteurized milk samples were autoclaved under conditions simulating retorting and then assayed for IGF-I content. These results were then compared to IGF-I concentrations measured in a commercial infant formula. The mean (±SEM) IGF-I concentrations in raw milk and pasteurized milk samples were 5.6 ± 0.56 and 8.2 ± 0.35 ng/ml, respectively. These same samples exposed to the heat treatment process for manufacturing infant formula contained concentrations of IGF-I of approximately 0.5 ng/ml and lower. The commercial infant formula also contained only trace amounts (approximately 0.7 ng/ml) of IGF-I. These results suggest that IGF-I is not destroyed by the pasteurization process, but the heating of milk for the preparation of infant formula denatures IGF-I, with only one-tenth of the concentration of the milk before heat treatment.

Although the pharmaceutical companies were not required to conduct studies with IGF-II, Monsanto conducted a study of milk residues to determine if IGF-II concentrations increased in rbGH-treated cows (*74*). Sixty-four lactating Holstein cows (21 primiparous and 43 multiparous) were used in the study; they received either 500 mg of rbGH in an oil-based prolonged-release formulation (approximately the proposed dose) or vehicle by intramuscular or subcutaneous injection at 14-day intervals. Treatments began at 60 ± 3 days postpartum and continued for at least 10 cycles. Composite milk samples from each cow were collected on day −7 of the pretreatment period and on day 7 of injection cycles 1 through

Table 6. Least squares means for the natural logarithm of and actual milk IGF-I concentrations and the numerical range of IGF-I concentrations after subcutaneous administration of 500 mg of rbGH every 14 days in a prolonged-release formulation. From (*72*) with permission ©1989 Monsanto Agricultural Company.

Sample	Treatment	Milk IGF-I concentration (ng/ml)		
		ln Concentration* (±SEM)	Antilog mean†	Range
Pretreatment	Control	1.62 ± 0.11	5.05	3.01–9.04
	500 mg rbGH	1.37 ± 0.11	3.95	0.84–7.53
Day 7	Control	1.15 ± 0.08	3.17	2.85–4.29
	500 mg rbGH	1.25 ± 0.07	3.50	1.56–7.05
Day 21	Control	1.21 ± 0.14	3.34	2.04–5.79
	500 mg rbGH	1.67‡ ± 0.14	5.33‡	2.67–8.83
Day 35	Control	1.21 ± 0.11	3.35	2.16–8.15
	500 mg rbGH	1.54‡ ± 0.11	4.68‡	3.23–7.38

*Least squares means ± SEM of least squares means. †Antilog of the log concentration. ‡These means are significantly different from the control values ($P < 0.05$).

Table 7. The effect of 500 mg of rbGH administered intramuscularly (i.m.) or subcutaneously (s.c.) on milk concentrations of IGF-I and IGF-II (least squares means ± SEM). From (*74*) with permission ©1989 Monsanto Agricultural Company.

Sampling period	Primiparous cows	Multiparous cows
	Milk IGF-I concentration (ng/ml)	
Overall cycle 1–10		
Control	3.5 ± 0.67	3.9 ± 0.39
I.m.	5.9* ± 0.59	5.9* ± 0.37
S.c.	6.1* ± 0.60	5.6* ± 0.39
	Milk IGF-II concentration (ng/ml)	
Overall cycle 1–10		
Control	106.6 ± 9.11	97.8 ± 6.21
I.m.	116.3 ± 8.47	107.2 ± 5.99
S.c.	116.4 ± 8.36	94.5 ± 5.95

*These means are significantly different from the control values ($P < 0.05$, protected t test).

10. There was no significant increase in milk IGF-II concentrations in any of the sampling periods ($P > 0.05$). However, the concentration of IGF-I in milk from the rbGH-treated cows was significantly increased across the ten injection cycles. The average increase in IGF-I concentration was 2.2 ng/ml in milk (Table 7).

It appears from these studies that IGF-I concentrations in the milk of rbGH-treated cows are increased above those concentrations found naturally in untreated cows. However, the data indicate that stage of lactation and parity also significantly influence IGF-I concentrations in milk. IGF-II milk concentrations, on the other hand, are not affected by rbGH treatment.

Conclusions

The data evaluated by the FDA document the safety of food products from animals treated with rbGH. Bovine GH is biologically inactive in humans; therefore, residues of bGH in food products would have no physiological effect even if absorbed intact from the gastrointestinal tract. The possibility that fragments of bGH produce metabolic effects in humans is not a basis for concern as it is unlikely that any active fragment could be produced in biologically significant amounts in the gastrointestinal tract. Very mild hydrolysis conditions are necessary to retain even the limited activity observed in test animals. No oral activity was found when rbGH was administered to rats at exaggerated doses. In addition, very limited residue studies suggest no significant increase in milk concentrations of bGH due to the treatment of dairy cows with rbGH. Furthermore, 90% of bGH in milk is destroyed upon pasteurization, and rbGH treatment appears to have no significant impact on the nutritional quality of milk.

The FDA concluded that an increase in growth factors secondary to rbGH treatment was unlikely to present any human food safety concerns. Nonetheless, the FDA felt it was important to establish the range of concentrations of growth factors after rbGH treatment and the potential for oral activity because of the widespread use of milk-based infant formulas. IGF-I was chosen as the growth factor for study because it is the major factor that mediates the effects of GH.

The oral toxicity studies demonstrated that rIGF-I was not active at doses up to 2 mg/kg per day in rats. Additional information, collected to resolve any concern for potential neonatal exposure to IGF-I, demonstrated that IGF-I is denatured by the process used to prepare infant formula, which eliminates any basis of concern for minor increases in IGF-I concentrations in milk. Although limited information is available about the concentration of IGF-I in human milk, the data indicate that the concentration of IGF-I found in milk from rbGH-treated cows is within the physiological range found in human breast milk. On the basis of this information, the FDA scientists concluded that the use of rbGH in dairy cattle presents no increased health risk to consumers.

REFERENCES AND NOTES

1. D. E. Bauman *et al.*, in *Proceedings of the 7th International Conference on Production Disease in Farm Animals*, F. A. Kallfelz, Ed. (Cornell Univ., Ithaca, NY, 1989), pp. 306–323.
2. FDA's Center for Veterinary Medicine has approved the use for human consumption of milk from investigational herds treated with rbGH, based on its conclusion about the safety of the drug. The Center has not yet taken final action on the pending new animal drug applications for use of rbGH in dairy cows.
3. Code of Federal Regulations, Title 21, Part 514, revised as of 1 April 1989.
4. Food and Drug Administration, *Fed. Regist.*, 52FR49583 (1986).
5. Code of Federal Regulations, Title 21, Part 58, revised as of 1 April 1989.
6. D. M. Matthews, *Physiol. Rev.* **55**, 537 (1975).
7. A. S. McNeish, *Ann. Allergy* **53**, 643 (1984); E. Lebenthal, P. C. Lee, L. A. Heitlinger, *J. Pediatr.* **102**, 1 (1983); E. Lebenthal and Y. K. Leung, *Pediatr. Ann.* **16**, 211 (1987).
8. I. G. Morris, in *Handbook of Physiology*, sect. 6, *Alimentary Canal*; vol. 5, *Bile; Digestion; Ruminal Physiology*; C. F. Code, Ed. (American Physiology Society, Washington, DC, 1968), pp. 1491–1512.
9. A. L. Warshaw, W. A. Walker, R. Cornell, K. J. Isselbacher, *Lab. Invest.* **25**, 675 (1971); W. A. Walker, R. Cornell, L. M. Davenport, K. J. Isselbacher, *J. Cell Biol.* **54**, 195 (1972).
10. A. L. Warshaw, W. A. Walker, K. J. Isselbacher, *Gastroenterology* **66**, 987 (1974).
11. R. J. Levinsky, *Proc. Nutr. Soc.* **44**, 81 (1985).
12. J. C. Leissring, J. W. Anderson, D. W. Smith, *Am. J. Dis. Child.* **103**, 160 (1962).
13. E. J. Eastham, T. Lichauco, M. I. Grady, W. A. Walker, *J. Pediatr.* **93**, 561 (1978).
14. D. M. Roberton, R. Paganelli, R. Dinwiddie, R. J. Levinsky, *Arch. Dis. Child.* **57**, 369 (1982).
15. M. C. Reinhardt, *Ann. Allergy* **53**, 597 (1984).
16. J. G. Lecce, *Environ. Health Perspect.* **33**, 57 (1979).
17. I. Jakobsson, T. Lindberg, L. Lothe, I. Axelsson, B. Benediktsson, *Gut* **27**, 1029 (1986).
18. T. Vukavic, *J. Pediatr. Gastroenterol. Nutr.* **2**, 248 (1983).
19. ———, *ibid.* **3**, 700 (1984).
20. A. de la Cruz *et al.*, *Fertil. Steril.* **26**, 894 (1975); I. Yamazaki *et al.*, *J. Takeda Res. Lab.* **38**, 64 (1977); J. A. Thomas and M. G. Mawhinney, in *Synopsis of Endocrine Pharmacology* (University Park Press, Baltimore, 1973), pp. 9–11; M. Amoss, J. Rivier, R. Guillemin, *Clin. Endocrinol. Metab.* **35**, 175 (1972); N. Nishi, A. Arimura, D. H. Coy, J. A. Vilchez-Martinez, A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **148**, 1009 (1975).
21. W. H. Daughaday, in *Endocrine Control of Growth*, W. H. Daughaday, Ed. (Elsevier, Amsterdam, 1981), pp. 1–24.
22. S. L. Davis, *J. Anim. Sci.* **66** (suppl. 3), 84 (1988).
23. O. G. P. Isaksson, A. Lindahl, A. Nilsson, J. Isgaard, *Acta Paediatr. Scand. Suppl.* **343**, 12 (1988).
24. S. A. Kaplan, *Am. J. Dis. Child.* **110**, 232 (1965).
25. J. L. Kostyo, *Metabolism* **23**, 885 (1974).
26. F. Matsuzaki and M. S. Raben, *Annu. Rev. Pharmacol.* **5**, 137 (1965); G. P. Talwar *et al.*, in *Recent Progress in Hormone Research*, G. Pincus, Ed. (Academic Press, New York, 1975), vol. 31, pp. 141–174; O. G. P. Isaksson, S. Edén, J. O. Jansson, *Annu. Rev. Physiol.* **47**, 483 (1985); R. D. Boyd and D. E. Bauman, in *Animal Growth Regulation*, D. R. Campion, G. J. Hausman, R. J. Martin, Eds. (Plenum, New York, 1989), pp. 257–293.
27. H. Green, M. Morikawa, T. Nixon, *Differentiation* **29**, 195 (1985).
28. E. Knobil and R. O. Greep, in *Recent Progress in Hormone Research*, G. Pincus, Ed. (Academic Press, New York, 1959), vol. XV, pp. 1–69.
29. M. S. Raben, *ibid.*, pp. 71–114.
30. N. Yamasaki, M. Kikutani, M. Sonenberg, *Biochemistry* **9**, 1107 (1970).
31. D. M. Bergenstal and M. B. Lipsett, *J. Clin. Endocrinol. Metab.* **20**, 1427 (1960).
32. J. B. Mills, S. C. Howard, S. Scapa, A. E. Wilhelmi, *J. Biol. Chem.* **245**, 3407 (1970).
33. C. A. Baile and D. H. Krestel-Rickert, *Feed Manage.* **36**, 26 (1985).
34. W. V. Moore, S. Draper, C. H. Hung, *Horm. Res.* **21**, 33 (1985).
35. M. Sonenberg *et al.*, in *Growth and Growth Hormone*, A. Pecile and E. E. Muller, Eds. (Excerpta Medica, Amsterdam, 1972), pp. 75–90.
36. L. Graf and C. H. Li, *Biochemistry* **13**, 5408 (1974).
37. K. Hara, C. J. H. Chen, M. Sonenberg, *ibid.* **17**, 550 (1978).
38. M. Sonenberg, M. Kikutani, C. A. Free, A. C. Nadler, J. M. Dellacha, *Ann. N.Y. Acad. Sci.* **148**, 532 (1968).
39. A. C. Nadler, M. Sonenberg, M. I. New, C. A. Free, *Metabolism* **16**, 830 (1967); M. Sonenberg *et al.*, *ibid.* **14**, 1189 (1965).
40. J. L. Nappier and G. A. Hoffman, unpublished report (Agricultural Research and Development Laboratories, Upjohn Co., Kalamazoo, MI, 1986); W. J. Seaman and P. J. Skinner, unpublished report (Pharmaceutical Research and Development, Upjohn Co., Kalamazoo, MI, 1986).
41. D. G. Serota, N. G. Phipps, R. D. Alsaker, J. E. Carter, B. Colpean, unpublished report (Hazleton Laboratories America, Inc., prepared for Monsanto Co., St. Louis, MO, 1984).
42. D. Richard, G. Odaglia, P. Deslex, unpublished report (Department of Product Safety Assessment, Searle Research and Development, prepared for Monsanto Co., St. Louis, MO, 1989).
43. J. E. Fischer, R. L. Depew, C. A. Lowe, J. Panfili, unpublished report (Agricultural Research Division, American Cynanamid Co., Wayne, NJ, 1984).
44. L. F. Fisher, unpublished report (Toxicology Division, Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN, 1985).
45. Note: Organs weighed included, at a minimum, the following: adrenals, brain, heart, kidneys, liver, ovary, spleen, and testes. Histopathological examination included, at a minimum, the following organs: adrenals, bone, bone marrow, brain, colon, duodenum, esophagus, heart, ileum, jejunum, kidneys, liver, lung, lymph nodes, mammary gland, ovary, pancreas, parathyroid, prostate, salivary gland, skeletal muscle, skin, spinal cord, spleen, stomach, testes, thymus, thyroid, trachea, urinary bladder, uterus, and all gross lesions.
46. B. K. Birmingham *et al.*, *J. Dairy Sci.* **71**, 227 (1988).
47. J. A. Moore *et al.*, *Endocrinology* **122**, 2920 (1988).
48. K. L. Simkins, in *Proceedings of the California Animal Nutrition Conference on Nutrient Partitioning*, E. Robb, Ed. (Fresno, California, 1987), pp. 36–45.
49. P. P. Groenewegen, B. W. McBride, J. H. Burton, T. H. Elsasser, *J. Nutr.* **120**, 514 (1990).
50. G. Van den Berg, in *Use of Somatotropin in Livestock Production*, K. Sejrsen, M. Vestergaard, A. Neimann-Sorensen, Eds. (Elsevier, Science, New York, 1989), pp. 178–191; R. J. Baer *et al.*, *J. Dairy Sci.* **72**, 1424 (1989).
51. A. J. D'Ercole, L. E. Underwood, J. J. Van Wyk, *J. Pediatr.* **90**, 375 (1977); R. W. Furlanetto, L. E. Underwood, J. J. Van Wyk, A. J. D'Ercole, *J. Clin. Invest.* **60**,

648 (1977).
52. J. F. Perdue, *Can. J. Biochem. Cell Biol.* **62**, 1237 (1984).
53. D. Maiter, L. E. Underwood, M. Maes, M. L. Davenport, J. M. Ketelslegers, *Endocrinology* **123**, 1053 (1988); K. C. Copeland, L. E. Underwood, J. J. Van Wyk, *J. Clin. Endocrinol. Metab.* **50**, 690 (1980); M. B. Grant *et al.*, *ibid.* **63**, 981 (1986).
54. J. J. Van Wyk, M. E. Svoboda, L. E. Underwood, *J. Clin. Endocrinol. Metab.* **50**, 206 (1980).
55. J. Zapf and E. R. Froesch, *Horm. Res.* **24**, 121 (1986).
56. K. Hall and V. R. Sara, in *Vitamins and Hormones* (Academic Press, New York, 1983), vol. 40, pp. 175–233; L. E. Underwood, A. J. D'Ercole, D. R. Clemmons, J. J. Van Wyk, *Clin. Endocrinol. Metab.* **15**, 59 (1986); R. C. Baxter, *Adv. Clin. Chem.* **25**, 49 (1986).
57. A. J. D'Ercole, A. D. Stiles, L. E. Underwood, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 935 (1984).
58. C. C. Orlowski and S. D. Chernausek, *Endocrinology* **123**, 44 (1988); J. Isgaard *et al.*, *ibid.* **122**, 1515 (1988); J. Isgaard, A. Nilsson, K. Vikman, O. G. P. Isaksson, *J. Endocrinol.* **120**, 107 (1988); N. L. Schlechter, S. M. Russell, E. M. Spencer, C. S. Nicoll, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7932 (1986).
59. J. Zapf, C. Hauri, M. Waldvogel, E. R. Froesch, *J. Clin. Invest.* **77**, 1768 (1986).
60. A. Skottner, R. G. Clark, I. C. A. F. Robinson, L. Fryklund, *J. Endocrinol.* **112**, 123 (1987).
61. E. Schoenle, J. Zapf, C. Hauri, T. Steiner, E. R. Froesch, *Acta Endocrinol.* **108**, 167 (1985).
62. K. Hall and V. R. Sara, *Clin. Endocrinol. Metab.* **13**, 91 (1984).
63. R. C. Baxter, Z. Zaltsman, J. R. Turtle, *J. Clin. Endocrinol. Metab.* **58**, 955 (1984).
64. A. N. Corps, K. D. Brown, L. H. Rees, J. Carr, C. G. Prosser, *ibid.* **67**, 25 (1988).
65. A. Honegger and R. E. Humbel, *J. Biol. Chem.* **261**, 569 (1986).
66. L. F. Fisher and E. L. Russell, unpublished report (Toxicology Division, Lilly Research Laboratories, Eli Lilly & Co., Indianapolis, IN, 1989).
67. J. B. Terrill, unpublished report (Hazleton Laboratories of America, Inc., prepared for Monsanto Agricultural Co., St. Louis, MO, 1989).
68. T. C. White *et al.*, unpublished report MSL 8671 (Monsanto Agricultural Co., St. Louis, MO, 1989).
69. R. J. Collier *et al.*, unpublished report MSL 8531 (Monsanto Agricultural Co., St. Louis, MO, 1988).
70. D. Schams and H. Karg, unpublished Elanco report, Eli Lilly & Co., dated 23 September 1988 (Institute of Physiology, Technische Universitat Munchen, D-8050 Freising-Weihenstephan, FRG, 1988); unpublished Elanco report, Eli Lilly & Co. (Institute of Physiology, Technische Universitat Munchen, D-8050 Freising-Weihenstephan, FRG, 1988).
71. S. R. Davis, P. D. Gluckman, A. M. Bryant, unpublished Elanco report, Eli Lilly & Co. (Ruakura Agricultural Centre, Hamilton, New Zeland, 1989).
72. T. C. White *et al.*, unpublished report MSL 8633 (Monsanto Agricultural Co., St. Louis, MO, 1989).
73. M. A. Miller *et al.*, unpublished report MSL 8673 (Monsanto Agricultural Co., St. Louis, MO, 1989).
74. M. A. Miller, T. C. White, R. J. Collier, unpublished report (Monsanto Agricultural Co., St. Louis, MO, 1989).
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Research Article

De Novo Design, Expression, and Characterization of Felix: A Four-Helix Bundle Protein of Native-Like Sequence

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The protein Felix was designed de novo to fold into an antiparallel four-helix bundle of specific topology. Its sequence of 79 amino acid residues is not homologous to any known protein sequence, but is "native-like" in that it is nonrepetitive and contains 19 of the 20 naturally occurring amino acids. Felix has been expressed from a synthetic gene cloned in *Escherichia coli*, and the protein has been purified to homogeneity. Physical characterization of the purified protein indicates that Felix (i) is monomeric in solution, (ii) is predominantly α-helical, (iii) contains a designed intramolecular disulfide bond linking the first and fourth helices, and (iv) buries its single tryptophan in an apolar environment and probably in close proximity with the disulfide bond. These physical properties rule out several alternative structures and indicate that Felix indeed folds into approximately the designed three-dimensional structure.

NEW TECHNIQUES IN MOLECULAR BIOLOGY HAVE OPENED up the potential for engineering the structural properties of proteins to desirable specifications. This possibility is being explored with mutagenesis procedures to alter the properties of existing structures and also by designing entire protein structures de novo.

De novo design represents an attempt to choose an amino acid sequence that is unrelated to any natural protein sequence, but that will fold into a desired three-dimensional structure. The principles and details of protein folding are not well enough understood to ensure the success of such attempts; nevertheless, we and others are tackling some of the simpler cases. The reason for taking such a drastic and uncertain step is that minor variants of natural proteins cannot be used to test determinants that control major topological differences in structure because of the obstacles presented by all those features of the protein that have been evolutionarily selected to fit the native structure. We believe there are important unsettled fundamental questions, and therefore are attempting de novo design

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