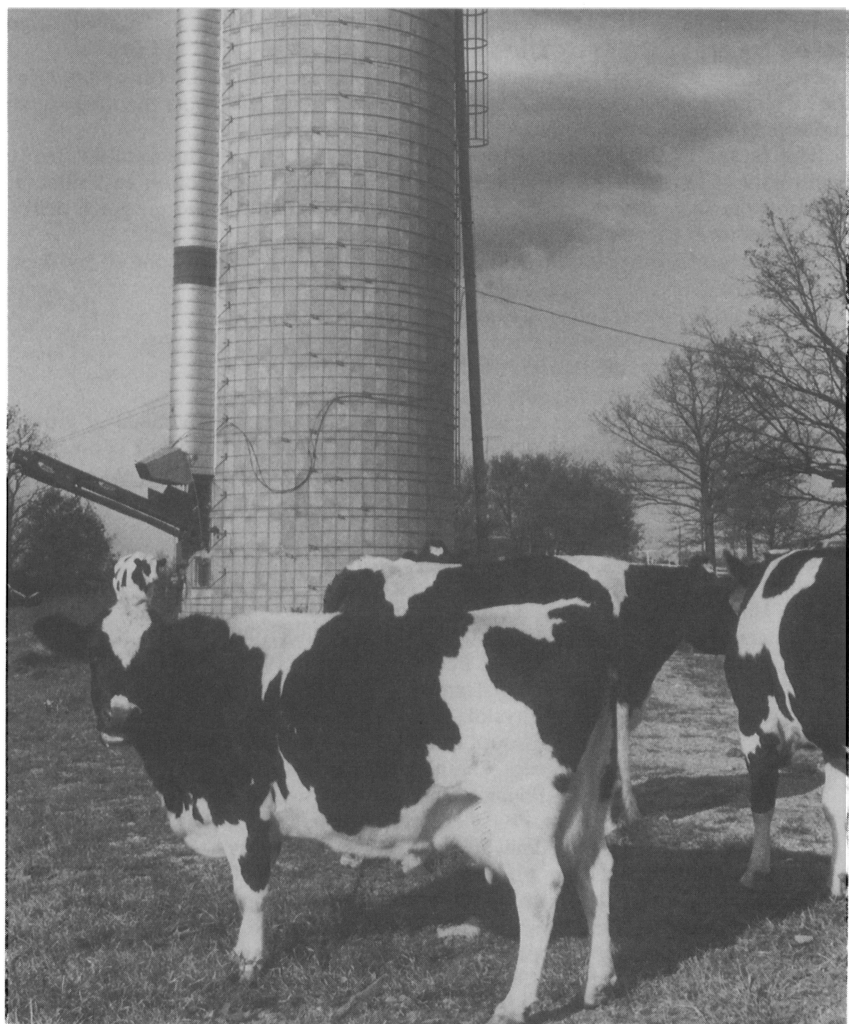


Physiologic and Therapeutic Roles of Somatotropin Effects in Adult Animals



Brody Memorial Lecture XXII

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Agricultural Experiment Station

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Establishment of Brody Memorial Lectureship

A committee was appointed by Dean Longwell to consider the possibility of creating a memorial for Samuel Brody. It was the opinion of the committee that a permanent lectureship would be most suitable if sufficient funds were obtained from friends, relatives, organizations and the University Faculty invited to contribute to this memorial.

Friends, relatives and organizations interested in recognizing Dr. Brody provided the initial funds, which were supplemented by a generous grant from the King Ranch and matching funds from the Alumni Achievement Funds.

The Board of Curators approved the establishment of the Samuel Brody Lectureship Fund in April 1959. Lectures have been held as often as sufficient income from the interest provided expenses and a small honorarium for a distinguished lecturer.

The current Brody Memorial Lectureship Committee was appointed by Dean Roger Mitchell:

Dr. Donald E. Spiers, Sigma Xi Representative

Dr. Harold D. Johnson, Department of Animal Sciences

Dr. B. Ann Becker, ARS Representative

Dr. Ralph R. Anderson, Gamma Sigma Delta Representative

The Committee welcomes additional contributions from individuals or groups in academia or industry. Such funds will be applied to the principal or endowment of the now-established Brody Memorial Lectureship Fund. Any increases in the endowment fund, or course, will allow lectures to be held more frequently.

Previous Brody Lectures:

- I. Max Kleiber, Dept. Animal Science, Univ. of Calif., Berkeley, 1960
- II. Knut Schmidt-Nielsen, Dept. Zoology, Duke Univ., 1961
- III. F. W. Went, Director, Missouri Botanical Garden, 1963
- IV. K. L. Baxter, Dept. Nutrition, Hannah Dairy Research Institute, 1964
- V. C. Ladd Prosser, Dept. Physiol., Univ. of Illinois, 1965
- VI. H. T. Hammel, Physiol. Group, John B. Pierce Found. Lab., 1966
- VII. H. N. Munro, Dept. Physiol. Chemistry, Mass. Institute of Tech., 1967
- VIII. James D. Hardy, Dept. Physiol., Yale University, 1968
- IX. Loren D. Carlson, Dept. Physiol., Univ. of Calif.-Davis, 1969
- X. R. L. Baldwin, Dept. Animal Science, Univ. of Calif.-Davis, 1971
- XI. John R. Brobeck, Dept. Physiol., School of Medicine, Univ. of Penn., 1972
- XII. Bruce A. Young, Dept. Animal Science, Univ. of Alberta, 1974
- XIII. D. E. Johnson, Dept. Animal Science, Colorado State Univ., 1975
- XIV. Albert L. Lehninger, Dept. Physiol. Chem., The Johns Hopkins School of Medicine, Baltimore, 1976
- XV. Henry A. Lardy, Dept. Biol. Science, Univ. of Wisc.-Madison, 1979
- XVI. H. A. Tucker, Depts. Dairy Science and Physiol., Mich. State Univ., 1981
- XVII. H. Russell Conrad, Dept. Dairy Science, The Ohio State Univ., 1982
- XVIII. David Robertshaw, Dept. Physiol/Biophysics, Colorado State Univ., 1984
- XIX. Allen Munck, Dept. Physiol., Dartmouth Medical School, 1986
- XX. Lawrence J. Machlin, Dir. Clinical Nutrition, Vitamins and Fine Chemicals Division, Hoffman-LaRoche Inc., 1988
- XXI. Keith W. Kelley, Dept. Animal Science, Univ. of Illinois, 1992

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Introduction

I am very honored to be invited to give the 22nd Brody Memorial Lecture. As a graduate student I was much influenced by the second, third and fourth lectures that I attended. Sam Brody, in his book "Bioenergetics and Growth," discussed many aspects of physiology including functions of hormones. In the early '40s, when he was writing the section on hormones, there was much to be discovered about growth hormone, as indicated by the quote below:

The above discussion does not answer the question as to whether or not there is anterior-pituitary hormone acting directly on body growth (independently of other target glands), as is believed by Evans. General agreement will probably be delayed until (1) the pure A.P. growth hormone (if such is present) is obtained, and (2) it is demonstrated that it does not act on other endocrines, or that it stimulates growth in the absence of other endocrines or hormone—perhaps an impossible demonstration since growth is an overall process, the resultant of innumerable interrelated factors. It therefore seems most logical to assume that growth is a function of many hormones, not of one specific growth hormone.

The most interesting agricultural feature of the claimed effect of A.P. "growth hormone" injection in young animals is the increase in growth rate and ultimate body size in general and protein retention in particular. The ultimate body size following A.P. "growth hormone" injection is apparently not larger than can be obtained by proper dietary means. The important claim, somewhat difficult to accept, is that on a given quantity of the same food, when the animals are said to be "pair fed" (see Ch. 20), animals treated with A.P. extract grow faster in weight (from Brody, 1945).

Clearly much information about growth hormone or somatotropin (ST) has been developed after this was written. The pure hormone has been produced in mass quantities, and many direct and indirect effects of ST have been demonstrated. Some of these effects are the subject of my lecture.

By 1946, the pituitary was known to synthesize a variety of peptide hormones including thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), lactogenic hormone (prolactin), adrenocorticotropin (ACTH) and growth hormone/somatotropin. The characterization of those peptides has taken many years, and only recently have the controls for their release been partially unraveled.

Somatotropin physiology and biochemistry has been of interest for many years. By 1886, it had been established that acromegaly was associated with malfunction of the pituitary and that hypophysectomy resulted in growth retardation. The presence of a growth-promoting hormone in the pituitary gland was recognized as early as 1912 by Cushing. In 1921, Evans and Long demonstrated a direct relationship between growth rate and the pituitary when they reported that an injection of a saline extract from bovine pituitary glands stimulated skeletal growth in rats. In 1927, Smith demonstrated that skeletal growth was stimulated by injection of bovine pituitary extract in hypophysectomized rats. He also reconfirmed the findings of Evans and Long that oral administration of ground bovine anterior pituitary tissue given to rats had no effect on growth. These early studies, for the most part, suggested that the pituitary gland secreted a substance that regulated somatic growth and development, and this substance became known as growth hormone or somatotropin.

Much of the actual experimental evidence of ST's physiological effects was demonstrated after the preparation of highly purified bovine ST by Li and Evans in 1944 and by Fishman et al., in 1947. The previous observations made with the use of crude extracts were confirmed with purified ST.

Recent advances in molecular biology have facilitated production of large quantities of recombinant hST. As a consequence, the production of pituitary hST for treatment in humans is no longer necessary. Goeddel et al., in 1979 first expressed hST in *E. coli* using recombinant DNA technology. Its growth-promoting properties were effective over a range of doses, and prospects of being clinically useful were evident. Recombinant hST has become an effective drug for treatment of ST deficiency syndromes. A recent interest is the biology of ST in adults, and consequently, new information about effects of ST treatment after linear growth has been completed is rapidly becoming available.

Somatotropin and Lactation

One physiological action of ST that has been known for many years is its influence on lactation. While some of the very early observations of activity were made in rabbits, Azimov and Krouze (1937) demonstrated in the 1930s using hundreds of cows that partially purified ST from bovine pituitaries would increase milk production. The response was replicated over the years and was also shown in goats, ewes, sows, etc. The mechanisms for these lactation responses are numerous (Bauman, 1990), and include indirect effects such as (1) enhanced nutrient supply to the mammary gland, (2) increased mammary secretory cell synthesis rates leading to increased milk secretion driven mainly by the increased insulin-like growth factor-I (IGF-I) and associated changes in IGF-I binding proteins and (3) favorable mineral kinetics, e.g., Ca. Direct actions of ST, such as the probable reduction in the gene expression of the membrane glucose transport protein Glut-4 in adipose tissue by ST, is an important repartition mechanism resulting in increased metabolites for milk secretion. Other ST actions on renal 25-hydroxyvitamin D-1-hydroxylase activity that lead to increased Ca and P absorption from the intestine and reabsorption of P in renal tubules provides a means for meeting the increased mineral demands associated with increased milk production. BST has been shown to enhance not only milk production but the efficiency of production primarily by reducing the nutrient proportion for maintenance and increasing that for milk production (Bauman, 1990).

Somatotropin and Reproduction

The use of ST in mature cattle is associated with several observable changes in reproductive parameters. Treatment of lactating or nonlactating cattle with ST increased follicular development (De La Sota et al., 1991; Gong et al., 1991). This was apparently not associated with an increase in circulating gonadotropins or gonadotropin receptor levels (Gong et al., 1991). However, plasma and follicular IGF-I concentrations were increased in cattle treated with bST (Lucy et al., 1992), indicating possible involvement of the somatomedin family in the increase in follicular development. Currently, it is not known whether bST acts locally to increase follicular IGF-I concentrations or whether IGF-I is taken up from plasma. However, the correlation between plasma and follicular IGF-I concentrations is relatively high (Spicer et al., 1992). The ST receptor is present in large but not small luteal cells and is undetectable in follicular tissue (Lucy et al., 1993). Collectively, these results support an indirect rather than local effect of ST on follicular development. However, there may be direct effects

of ST on the corpus luteum. As stated previously, the large luteal cell contains abundant message for the ST receptor (Lucy et al., 1993); and treatment of cattle with bST increased the size of the corpus luteum (Lucy et al., 1992). However, effects of ST on the concentration of progesterone in plasma have been equivocal with reports of increased progesterone in some studies and no change in others (Gallo and Block, 1991; Cole et al., 1991). This may be related to the stage of the estrus cycle in which animals were started on ST, but this hypothesis has not yet been tested.

Other effects of ST treatment on lactating cattle include delayed conception in controlled studies where the breeding interval was extended (Cole et al., 1991) and a slight increase in multiple births in some herds. The mechanism responsible for the increase in twinning may be related to the increase in follicular development, although this has not yet been tested. The delay in conception in ST-treated animals is directly related to the adjustment period required for animals to shift their feed intake and energy balance to the increase in milk yield. The resulting intercalving interval was approximately 13 months. No effects of ST on fetal loss were detected (Cole et al., 1991).

In recent experiments with pST and gestating gilts, both embryo survival and muscularity of embryos and pigs were increased (Kelley et al., 1992 and 1993). Gilts were treated with pST during days 28-39 of gestation, a period during which embryonic resorption and formation of primary myofibers occurs. On day 40, treated gilts, compared to controls, had 2.3 more embryos per litter and increased crown-rump lengths. The embryos showed evidence of changes in myogenic gene activity associated with pST treatment. Pigs from gilts similarly treated compared to pigs from control gilts had heavier semitendinosus muscles and larger longissimus muscle cross-sectional areas. The authors concluded that early embryonic development processes are sensitive to manipulation through treatment of gestating gilts.

Other ST Effects in Adult Mammals

Somatotropin secretion diminishes after maturity, and thus many of the activities of ST in juveniles are greatly curtailed in adults. In terms of nutrient partitioning, older animals, including people, have increased fat deposition and decreased muscle mass (Rudman et al., 1991). This is reversible, at least in part, by ST supplementation (Christiansen et al., 1991). Bone mineral density is clearly decreased in long-standing ST deficiency in man (Bouillon, 1991). There is evidence that bone content and density are enhanced by supplemental ST in some but not all ST-deficient patients. Adult dogs treated with large doses of ST showed increases in bone formation at both peritoneal

and endosteal surfaces, resulting in a net increase of total skeletal mass (Harris et al., 1972). ST treatment also increased bone mass in obese Zucker rats (Martin et al., 1989). The potential use of ST in fracture repair is being studied because ST stimulates bone cell growth and function (Bak and Andreassen, 1991). This application and one for treating osteoporosis are likely to provide benefits where ST deficiency is present due to pituitary disease or as a part of the aging process. It has been concluded that other factors must contribute to the age-related decline in fracture repair because ST supplementation only partially replenishes this function to that of young animals.

Somatotropin is now known to have immunomodulatory effects. Susceptibility to infections, neoplastic diseases and autoimmune diseases may be influenced by ST status. Part of the aging process includes a diminution of immune function and is associated with atrophy of the thymus gland (Khansari and Gustad, 1991). Macrophages have been primed by ST for production of superoxide anions (Kelley, 1989). Furthermore, polymorphonuclear neutrophil (PMN) adhesiveness may be stimulated by ST (Wiedermann et al., 1991). Supplemental ST in healthy adult humans increased natural killer cell activity indicated by an increase in the percent specific lysis of K562 tumor target cells.

Treatment of adult animals with ST has led to some positive effects on immune function. Bovine ST treatment of 3- to 5-year-old dogs resulted in regeneration of thymic morphology, presumably, in part, through the action of increased production of thymulin (Goff et al., 1987). Enhanced *in vivo* host protection against *Salmonella typhimurium* in rats treated with ST was reported by Edwards et al. (1989). Immune function and life expectancy of Balb/c mice treated with a low dose of hST was studied by Khansari and Gustad (1991). The mice were treated with ST starting at 17 months of age after signs of senescence and casual death occurred. There were no significant differences between cytokine production (IL-1, IL-2, TNF and IgG) in the young and ST-treated groups, but the old, untreated group had diminished IL-2. ST-treated mice had higher IL-1 and TNF production rates and significantly reduced mortality compared with the old, untreated mice. The authors postulated that the apparent prolongation of the mean life expectancy by ST treatment was due to a delay or prevention of age-associated disorders. It is not clear yet whether the responses observed were specific for the strain of mice selected for the study.

Summary

Only recently has an interest developed in the physiologic importance of ST in adults, and therefore, little is known about effects of ST treatment after linear growth has been completed. One exception to this is the well-established enhancement of lactation in cows. Increased milk yield is now known to be a typical response to exogenous ST in mammals, and higher-producing cows have greater secretion rates of ST. Aside from the effects on lactation and reproduction, many of the other actions of ST in adult mammals are suppression or regression of processes associated with aging. ST and insulin-like growth factor-I concentrations diminish with age, and these changes may permit initiation of many processes of aging. Examples of the effects of ST supplementation in aging animals and humans include increased muscling, reduced adipose tissue, liver regeneration and increased basal metabolic rate. ST stimulates (1) bone turnover via increased osteoblast number and function and, thus, bone resorption and (2) renal 25-hydroxyvitamin D-1 α -hydroxylase activity, thus increasing Ca and P absorption from the intestine and the reabsorption of P in renal tubules. The healing of both bone and certain types of wounds are enhanced by ST treatment in adults. ST has been shown in adult animals to cause increased thymulin and the reversal of the thymus atrophy associated with aging. In aging mice, a long-term, low-dose therapy with ST improved immune function and prolonged life span. Although there are no reported direct side effects, at least in lactating cows, it is possible that, in man, potential undesirable side effects associated with ST therapy, such as hyperglycemia and increased organ size, can be controlled by careful management of ST dose. Presumably there are other important factors in the aging process, but it seems likely that replacement therapy to maintain the higher ST levels present during early adulthood will diminish the onset of certain aspects of aging.

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Biography of Clifton A. Baile

Clifton A. Baile, Distinguished Fellow, Head of Animal Sciences/Molecular Biology Division, Monsanto Agricultural Company, St. Louis, Mo.

Dr. Baile was born in Warrensburg, Mo., on February 8, 1940. He graduated with a bachelor of science degree from Central Missouri State College in 1962 and earned his Ph.D., in nutrition from the University of Missouri in 1965 under the direction of Dr. William Pfander. His dissertation research on the regulation of feed intake in domestic animals has remained a lifelong interest. During his studies at the University of Missouri, Dr. Baile was awarded a Ralston Purina Fellowship.

Dr. Baile accepted an NIH postdoctoral fellowship in the School of Public Health at Harvard University in 1964, and there he began pioneering techniques with Dr. Jean Mayer for the study of the hypothalamic regulation of feed intake in collaboration. He was appointed instructor of nutrition in 1966 and assistant professor in 1968. That same year, Dr. Baile joined Smith Kline Animal Health Products, where he was manager of neurobiological research. At the same time, he held the position of research associate at the Monell Chemical Senses Center and lecturer in nutrition at the University of Pennsylvania.

In 1975, Dr. Baile, accepted a position as associate professor of nutrition at the University of Pennsylvania, where he was promoted to professor in 1979. He joined the Nutrition Chemicals Division of the Monsanto Agricultural Company in 1982 as a senior fellow, where he implemented a program in new product identification. In February 1986, he was named director of the New Product Identification Department, and in March 1987, he was appointed senior fellow and director of research and development of the Animal Sciences Division. In April 1988, Dr. Baile was appointed a distinguished fellow, one of only four scientists to hold this distinction in the approximately 3,500-member scientific community at Monsanto.

Since 1985, Dr. Baile has held a position as adjunct professor of nutrition at the Washington University School of Medicine, where he has had a laboratory with NIH funding and has continued his investigation into the hypothalamic regulation of feed intake. In 1986, he was appointed adjunct professor of nutrition in the Animal Sciences Department at the University of Missouri.

Dr. Baile's contribution to the literature on the relationship between growth, lactation and the control of feed intake includes approximately 225 journal articles, reviews and chapters and a similar number of abstracts for scientific meetings. Dr. Baile received the American Feed Manufacturers Award for his research in dairy nutrition in 1979 and was the recipient of the 1989 ASAS Animal Growth and Development Award.



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