all organs except the BC the increases were significantly higher in females given larger doses of AJH.

Thus, the data showed that the normal posteclosion growth of both the BC and RG occurring in intact female adult Monarchs was prevented by procedures removing the source of JH, i.e., by allatectomy or neck-ligation. In addition, the data showed that in females lacking the source of JH apparently normal growth of both glands was produced by injections of material (AJH) known to possess high JH activity in this species[1-3]. Finally, the BC and RG changes occurring in response to AJH seemed to be dose-related, and they clearly paralleled those observed in 2 female organs (the OV and CG) known to be regulated by JH[1-3]. It therefore appears that the posteclosion growth of the BC and RG of this species, like that of the OV and CG, is regulated by JH. Apparently, the relative importance of this regulatory mechanism declines with age, since studies on female Monarchs several weeks or months after eclosion have failed to provide conclusive evidence for JH mediated regulation of the BC and RG[3].

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Prolactin and growth hormone do not interfere with the response of mouse testes to hCG in vitro[1]

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Summary. In incubations of decapsulated mouse testes with a maximally stimulating dose of hCG, the accumulation of testosterone was not affected by the addition of PRL at concentrations from 0.1 to 50 µg/ml or GH at concentrations from 0.5 to 25 µg/ml.

Hypogonadism in hyperprolactinemic men is most likely due to inhibition of LHRH release and consequent gonadotropin deficiency[2,3], but the possibility of direct inhibitory effects of PRL on testicular steroidogenesis has not been eliminated. Experimental induction of hyperprolactinemia in mice is associated with a reduction in the number of testicular LH receptors and in the responsiveness of the testes to hCG stimulation in vitro[4]. These effects are thought to be due to a chronic elevation of peripheral LH levels which accompanies hyperprolactinemia in this species, but the possibility remains that PRL may also exert direct inhibitory effects on the testes. The present work was undertaken to determine whether the acute steroidogenic response of the testes to gonadotropin stimulation in vitro is affected by the presence of PRL in the incubation media. Because of the overlap of biologic activities of PRL and GH, the effects of the latter hormone in the same system were also examined.

The experiments were conducted using procedures originally developed by Dufau et al.[5] and by Van Damme et al.[6]. Adult random-bred mice were killed by cervical dislocation; testes were removed, decapsulated, and incubated for 4 h in 2 ml Krebs Ringer bicarbonate buffer containing glucose (1 mg/ml) and various doses of hCG (A.P.L. chorionic gonadotropin, Ayerst Laboratories) in a Dubnoff metabolic shaker at 32 ± 1 °C (under O_2:CO_2 (95:5). Ovine PRL (NIH-P-S12) and ovine GH (NIH-GH-S11) were dissolved in physiologic saline containing a few drops of 0.1 N NaOH, diluted and added to incubations to achieve the desired concentrations. Control incubations contained contralateral testes from the same mice and identical amounts of hCG, saline and NaOH. Testosterone (T) radioimmunoassays were performed directly on aliquots of the incubation medium[6-8].

In pilot studies, addition of 0.1–10 µg oPRL/ml to mouse testes incubated with 12.5 mIU hCG/ml caused a significant and dose-related increase in the accumulation of T in the media. However, addition of 20 ng FSH plus 5 ng LH/ml (maximal amounts of FSH and LH which could be present in 1 µg of NIH-P-S12) also caused a pronounced stimulation of T accumulation. In order to eliminate the influence of gonadotropins present in the oPRL preparation, an approach similar to that developed by Johnson and Ewing[9] was utilized, namely all further experiments were conducted in the presence of maximally stimulating amounts of hCG. The dose of hCG maximally effective in this system was determined to equal 100 mIU hCG/ml. In the presence of this dose of hCG, addition of oPRL at concentrations of 0.1–50 µg/ml had no significant effect on T production (table). Similarly, addition of 0.5, 5 or 25 µg GH/ml to incubations containing 100 mIU HCG/ml had no effect on the accumulation of T in the media. In additional experiments, effects of 2.5 µg oPRL/ml were examined in the presence of a higher and a lower dose of hCG. In incubations containing 50 mIU hCG/ml, this dose of oPRL caused a significant stimulation of T accumulation (504 ± 47 vs 818 ± 76 ng/ml; $p < 0.001$; $n = 18$), while in the presence of 200 mIU hCG/ml it was without effect (966 ± 69 vs 1065 ± 100 ng/ml; $n = 9$).

Thus the present findings argue against the possibility that alterations of testicular function in hyperprolactinemic

Effects of prolactin (PRL) and growth hormone (GH) on the production of testosterone by decapsulated mouse testes incubated with 100 mIU of human chorionic gonadotropin (hCG)/ml. Contralateral testes from the same animals incubated with hCG alone served as controls. The results represent mean (± SE) concentration of testosterone in the medium at the end of a 4-h incubation

Treatment	Concentration of testosterone (ng/ml)		
	Number	Controls	Treated
PRL; 0.1 µg/ml	12	1240 ± 105	1232 ± 67
PRL; 0.5 µg/ml	12	1201 ± 86	1167 ± 87
PRL; 2.5 µg/ml	15	923 ± 126	1026 ± 122
PRL; 5.0 µg/ml	27	843 ± 99	1013 ± 79
PRL; 25.0 µg/ml	8	1867 ± 208	1973 ± 164
PRL; 50.0 µg/ml	6	1384 ± 58	1396 ± 151
GH; 0.5 µg/ml	6	1061 ± 123	998 ± 104
GH; 5.0 µg/ml	6	1120 ± 99	1094 ± 117
GH; 25.0 µg/ml	9	1369 ± 153	1465 ± 113

males are due to a direct action of elevated PRL levels on the testis. Our results probably cannot be discounted as an artifact of the in vitro system, since both PRL and GH can affect functioning of cultured cells and incubated tissues[10-12]. However, specific PRL binding sites have been demonstrated in the interstitium of the rat testis[13] and PRL is capable of inhibiting steroidogenesis in long-term cultures of both testicular[14] (and G.F. Erickson, personal communication) and ovarian cells[10].

Another implication of the present findings may be worthy of note. Stimulation of T synthesis by dispersed mouse Leydig cells is widely used as a bioassay of LH[15,16]. Our results suggest that the results of this assay are unlikely to be affected by PRL or GH present in serum samples.

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DISPUTANDUM

The stereoselectivity of alcohol dehydrogenases: A stereochemical imperative?[1]

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Summary. The stereoselectivity of NAD^+-dependent alcohol dehydrogenases (transfering either the pro-R or pro-S hydrogen of NADH) correlates with the thermodynamic stability of their substrates, and appears to reflect evolutionary pressure to adjust in the active site the conformation of NADH so as to match the cofactor's reducing power to the oxidizability of the substrate. A requirement that the free energies of protein-bound intermediates be matched suggests a new approach for understanding catalysis and evolution in enzymes.

Stereoselective transfer of hydrogen to and from nicotinamide cofactors is the best studied example of the power of enzymes to make stereochemical choices. Westheimer, Vennesland and coworkers[2] first demonstrated this selectivity in the early 1950's, showing that dehydrogenases acting on NADH occur in 2 stereochemically distinct classes. Members of 1 class catalyze the transfer of the pro-R (A) hydrogen; member of the 2nd class catalyze the transfer of the pro-S hydrogen. More recently, work on well over a 100 additional dehydrogenases has shown that roughly half of all dehydrogenases belong to the 1st class and half to the 2nd[3,4]. The large body of data for dehydrogenases has prompted many authors[4-7] to attempt to discern a pattern in the stereopreferences of these enzymes, Hoping to understand why a particular dehydrogenase belongs to a particular class, several investigators have formulated a variety of 'rules' regarding the stereochemical preferences of dehydrogenases[4-7]. However, most of the 'rules' have exceptions, and some appear decidedly ad hoc; none have a clear mechanistic basis. Thus, none appear to have been accepted as part of a general explanation for the stereopreferences of dehydrogenases. Still others have concluded that the data contain no pattern whatsoever, that no mechanistic explanation exists, and that the stereopreferences are 'random'[8]. Thus, despite a quarter century of speculation, the mechanistic basis for the different stereochemical behavior of dehydrogenases remains a mystery.

Stereochemical ambivalence in dehydrogenases is unusual, since stereochemical uniformity seems to be the rule for most classes of enzymes. These uniformities have been analyzed recently by Hanson and Rose[8], who argued that uniform stereochemical preferences displayed by the members of many classes of enzymes catalyzing similar reactions might reflect the existence of a 'mechanistic imperative' in these enzymes. For chemical reasons inherent in the nature of the reaction being catalyzed, a single mode of catalysis producing a certain stereochemical outcome is presumed to be more efficient than alternative modes that produce other stereochemical outcomes. Because an organism possessing highly efficient enzymes is more likely to survive than a competing organism possessing less efficient enzymes, Hanson and Rose[8] argued that evolutionary processes may have forced the selection of only those enzymes that are optimal catalysts, and thereby have selected only those enzymes making the optimal stereochemical choice.

The stereochemical similarities within a class of enzymes can be quite striking. For example, all enzymes using pyridoxal cofactors shuttle a proton by attacking C-4′ of the pyridoxal group from the si face. All amino acid decarboxylases proceed with retention of configuration. Of course, these uniformities can possibly be understood in 'historical' terms; if all of the members of these classes of enzymes have evolved from a common ancestral protein that catalyzed a primeval prototypical reaction, the stereochemical preferences of the ancestor may have been conserved. Nonetheless, Hanson and Rose[8] suggested a number of chemical explanations for why one stereochemical outcome might be catalytically superior to an alternative outcome. In the case of dehydrogenases, where non-uniformity is the rule, the arguments of Hanson and Rose force one to the