serum. Statistical analysis of the LD_{50} data showed that KLH, 315-KLH, and 4C11 did not significantly increase the resistance of the mice to pneumococcal infection. The LD_{50} for mice primed with PC-KLH and 4C11-KLH, however, was almost 100 times greater than that for normal mice, indicating that an idiotope vaccine can be as effective in eliciting protection as the conventional vaccine based on nominal antigen (PC-KLH) (*14*). The high titers of antibody to PC in mice primed with PC-KLH and 4C11-KLH (Table 1) correlated with the increased protection seen in the LD_{50} experiments.

These results show that a monoclonal anti-idiotope vaccine is effective in protecting mice against a lethal bacterial infection. The functional significance of an idiotope-based vaccine has been suggested (*15–17*) and was demonstrated against African trypanosomiasis (*18*). However, that work differed from ours in that an allogeneic idiotypic antiserum preparation was used as an immunogen.

The antibody to the idiotope alone was not effective in protecting against *S. pneumoniae* but had to be coupled to a protein carrier. Presumably, T-helper cells needed for the serum antibody response are activated by the conjugate of idiotope antibody and carrier. The relative inefficiency of the internal idiotope alone in inducing protection can be predicted from the immune network hypothesis, which dictates that the internal antigens must remain silent for homeostasis of the immune system.

Internal antigen images provide the means to construct novel vaccines that may prove superior to conventional ones. The most promising application of idiotope vaccines is in the immunotherapy of tumors, which is hampered by the unavailability of pure tumor antigens and the preexisting tolerance to tumor-associated antigens.

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Essential Role of Insulin in Transcription of the Rat 25,000 Molecular Weight Casein Gene

Abstract. *Insulin is essential for the accumulation of rat casein messenger RNA (mRNA) in the presence of glucocorticoid and prolactin. The accumulation of certain mRNA's in other tissues has also been linked to insulin action. The present study shows that the accumulation effect on the 25,000 molecular weight rat casein mRNA does not reflect stabilization of the transcript by insulin. Rather, insulin is essential for its synthesis in the presence of glucocorticoid and prolactin.*

In a review of studies on the effects of insulin on RNA (*1*), most of the reports showed that the levels of certain proteins are elevated by insulin and that these effects are prevented by actinomycin D, an inhibitor of RNA synthesis. Insulin-dependent gene expression has also been shown to be related to changes in the accumulation of the corresponding messenger RNA's (mRNA's) (*2–6*). The murine mammary gland is a model useful for studying the role of insulin in relation to mRNA. Insulin (*7*), glucocorticoid (*8*), and prolactin (*7*) are essential for the accumulation of casein mRNA [molecular weight, 25,000 (25K)] in rat mammary explants. Although insulin-like growth factor II (IGF II) [multiplication stimulating activity (*9*)] and IGF I [somatomedin C (*10*)] can maintain the tissue in the synthetic medium as well as insulin, they do not mimic effectively the action of insulin either on casein synthesis or on the accumulation of casein mRNA. This suggests that the effect of insulin on casein gene expression is hormone-specific and does not involve interaction of the hormone with receptors for IGF I or II.

Increased accumulation of mRNA can result from stabilization of the transcript or an increased rate of transcription or both. In experiments to determine the effect of insulin on these effectors of the accumulation of rat 25K casein mRNA, rat mammary explants were cultured in Medium 199 to which four agents were added in various combinations. Serum was added to all the systems because it can maintain the tissue in the absence of insulin (*7*). In those instances in which it was of interest to operate in the absence of serum-derived prolactin, antiserum to prolactin was used. Antiserum to insulin was added when it was of interest to operate in the absence of serum-derived

Table 1. Effect of insulin (I) and prolactin (P) on the accumulation of rat 25K casein mRNA. Mammary explants from rats at day 12 of pregnancy were cultured in 2.5 ml of Medium 199 as described (*17*). All tissue was first cultured with lamb serum (20 percent) and cortisol (50 ng/ml) for 2 days (SF). Culture was then continued with added prolactin (1 μg/ml) or insulin (10 ng/ml) or both for up to 3 days more. At the start of the second culture period, antiserum to insulin (1 μl per dish) was added to all systems to which insulin had not been added, and antiserum to ovine prolactin (2 μl per dish) was added to all systems to which prolactin had not been added. In all cases, the SF medium was treated with the appropriate antiserum for 12 hours before tests. A 1-μl sample of insulin antiserum precipitated 30 ng of insulin, and a 2-μl sample of prolactin antiserum precipitated 150 ng of prolactin. Total RNA was isolated from the explants by the guanidinium–cesium chloride method (*18*). The 25K casein mRNA component was determined by the DBM disk method (*8*). Total RNA (1 μg) bound to DBM disks was hybridized with nick-translated ^{32}P-labeled p530–25K casein DNA (480,000 count/min) at 42°C for 18 hours. Each value represents the mean of triplicate determinations ± standard error. Comparable results were obtained in three similar experiments. Zero time indicates the freshly isolated tissue; N.D., not detectable.

Conditions	Day	Hybridization*
Zero time		7183 ± 227
SF	2	174 ± 8
	5	N.D.
SF → SFP	5	26 ± 14
SF → SFI	5	N.D.
SF → SFPI	3	404 ± 43
	4	768 ± 98
	5	2293 ± 31

*Amount of ^{32}P-labeled p530–25K casein DNA hybridized, expressed as counts per minute per microgram of RNA.

insulin. Cortisol was also added to all the systems to satisfy the glucocorticoid requirement of the system (*8*). The role of glucocorticoid was not studied further. Prolactin was added to some of the systems because it is necessary for the accumulation of rat 25K casein mRNA (*7*). Our primary objective was to determine the effects of insulin on the transcription and half-life of the 25K casein mRNA. In previous studies insulin was present in the culture medium, but its contributions to the system were not considered (*11*).

After rat mammary explants are exposed to step-down conditions (incomplete complement of hormones) in the presence of fetal bovine serum, enhanced accumulation of 25K casein mRNA requires the addition of both insulin and prolactin in the presence of cortisol (*7*). In the present study, it was of interest in certain instances to operate in the absence of serum-derived prolactin. Since antiserum to ovine prolactin, but not to bovine prolactin, is commercially available, lamb serum was used instead of fetal bovine serum. For this reason, the 25K casein mRNA accumulation responses in the presence of lamb serum were compared with those reported earlier (*7*). Step-down for 2 days in the presence of lamb serum and cortisol resulted in a decline from the initial level of the mRNA (Table 1). The decline was even greater after an additional 3 days of incubation with serum, cortisol, and prolactin, whereas no detectable mRNA remained after an additional 3 days with serum, cortisol, and insulin. Enhancement above the level observed after the 2-day step-down occurred only when both insulin and prolactin were added—that is, in the system containing serum, cortisol, insulin, and prolactin. Autoradiographic confirmation of these conclusions is presented in Fig. 1. It may be noted that the 25K casein mRNA, when present, migrated as a band of material about 1.3 kb (kilobases) in length compared to denatured DNA standards. Thus lamb serum and fetal bovine serum (*7*) permit similar responses.

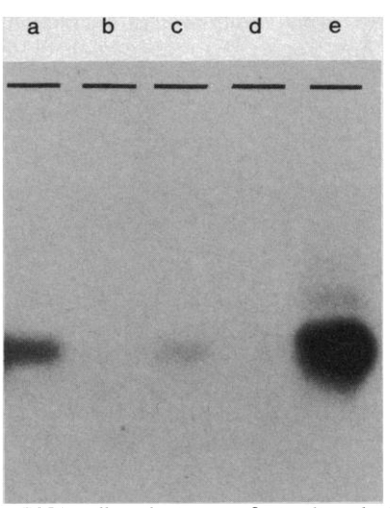
In the studies on the half-life of the 25K mRNA, the tissue was first exposed to step-down conditions in the presence of serum and cortisol for 2 days, exposed to insulin or prolactin or both for 1 hour, and subjected to a 30-minute uridine pulse-chase (exposed to ^{3}H-labeled uridine for 30 minutes and then to unlabeled uridine). The half-life of the 25K casein mRNA in the presence of serum and all three hormones was about 24 hours (Fig. 2). In the absence of prolactin the half-life was only about 6 hours. Therefore prolactin exerts a marked stabilizing effect on the mRNA, as reported earlier (*11*). The half-life of the mRNA was about 20 hours in the serum-cortisol-prolactin system compared to 24 hours when insulin was also present. Thus, insulin has little influence on the half-life even though it is essential for accumulation of the 25K mRNA (Table 1).

In experiments to determine the role of insulin in the expression of the 25K casein gene (Table 2), nuclei were isolated and pulsed with ^{32}P-labeled uridine triphosphate after the explants had been

Table 2. Effect of insulin and prolactin on transcription of the 25K casein gene. Cultures were conducted as described in the legend to Table 1. After day 5 of culture, nuclei were isolated from mammary explants by homogenization in buffer containing sucrose (2*M*), $MgCl_2$ (5 m*M*), tris-HCl (10 m*M*, *p*H 7.5), and Triton X-100 (0.2 percent) and then were centrifuged at 20,000*g* for 1 hour. Transcription of the 25K casein gene was assayed by pulsing with ^{32}P-labeled uridine triphosphate as described (*19*). The reaction was terminated by addition of deoxyribonuclease I (10 μg/ml) and proteinase K (100 μg/ml). After addition of 25 μg of yeast RNA, ^{32}P-labeled RNA was isolated and hybridized (~2 × 10^6 count/min) to p530–25K casein DNA bound to DBM disks as described in the legend to Fig. 2. Each value represents the mean ± standard error of triplicate determinations. Similar results were obtained in three experiments. Zero time indicates nuclei from freshly isolated tissue; N.D., not detectable.

Conditions	Transcription	
	Total*	25K mRNA†
Zero time	1.05	5752 ± 1014
SF	0.98	N.D.
SF → SFP	1.21	N.D.
SF → SFI	1.31	N.D.
SF → SFPI	1.95	6979 ± 88

*Expressed as counts per minute × 10^{-6} per 25.6 μg of DNA. †Expressed as parts per million of total labeled RNA ± standard error.



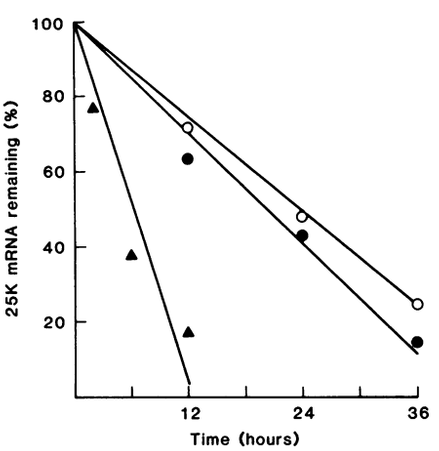


Fig. 1 (left). Autoradiography of accumulated 25K casein mRNA. Explants were cultured as described in the legend to Table 1. Total RNA was isolated and subjected to electrophoresis (2 μg per lane) in formaldehyde-agarose (1 percent) mini gel (*14*). The RNA was transferred to Gene Screen Plus membrane in 10× SSC (standard saline citrate), and hybridized in a solution containing nick-translated ^{32}P-labeled p530–25K casein DNA (3 × 10^6 count/min per milliliter), dextran sulfate (10 percent), NaCl (1*M*), tris-HCl (50 m*M*, *p*H 7.5), sodium dodecyl sulfate (1 percent), and yeast RNA (200 μg/ml) at 65°C. After 16 hours the membrane was washed according to the manufacturer's protocol and subjected to autoradiography for 4 hours with intensifying screens at −70°C. Abbreviations: S, lamb serum; F, cortisol; P, prolactin; I, insulin. (Lane a) SF, 2 days of culture; (lane b) SF, 5 days of culture; (lane c) SF for 2 days, then SFP for 3 days; (lane d) SF for 2 days, then SFI for 3 days; (lane e) SF for 2 days, then SFPI for 3 days. Fig. 2 (right). Effect of insulin and prolactin on the half-life of the rat 25K casein mRNA. All explants were first cultured with serum and cortisol for 2 days, as described in the legend to Table 1, then insulin, prolactin, or insulin plus prolactin was added. After 1 hour the explants were transferred to Waymouth's medium containing 20 percent lamb serum, appropriate hormones, and ^{3}H-labeled uridine (1 mCi/ml). After a 30-minute pulse, explants were washed and cultured in medium containing serum, cortisol, and 20 m*M* uridine, insulin, or prolactin or both, and antiserum to prolactin or to insulin as indicated in the legend to Table 1. Total ^{3}H-labeled RNA was isolated from explants by the guanidinium–hot phenol method (*15*), washed with 3*M* sodium acetate, and hybridized to excess p530–25K casein DNA bound to diazobenzyloxymethyl (DBM) disks (10 μg DNA per disk) (*16*). Briefly, ^{3}H-labeled RNA (3 × 10^6 to 4 × 10^6 count/min) was hybridized in a solution containing formamide (50 percent), 4× SSC, SDS (1 percent), and yeast RNA (200 μg/ml) at 37°C for 3 days. The DBM disks were washed, ^{3}H-labeled RNA was eluted with NaOH and neutralized, and radioactivity was determined in a liquid scintillation counter. Hybridizable label in the 25K casein mRNA, expressed as counts per minute per 10 mg of explant, was determined immediately after the pulse (time zero) and at the times indicated. Time-zero values were 3630, 3670, and 6350 count/min per 10 mg of explant for the SFP (●), SFI (▲), and SFPI (○) systems, respectively. Similar results were obtained in three experiments.

cultured for 5 days. The results showed that the initial transcriptional ability is lost at this time in all the incomplete systems. This differs, as shown in Fig. 2, from the situation after only 2 days in the incomplete systems; under these conditions some of the initial transcriptional ability was still retained in the incomplete systems. After 5 days of culture the only nuclei capable of transcribing the 25K casein gene were those isolated from tissue that had been exposed to all three hormones (Table 2). These nuclei had a high level of this transcriptional activity; α-amanitin (5 μg/ml) eliminated the activity (not shown). Thus, transcription of the 25K casein mRNA in mammary cells requires both insulin and prolactin. In contrast, synthesis of total RNA (derived largely from epithelial nuclei) was hardly affected by the step-down conditions.

Earlier work (*11*) has shown that prolactin has a dual role in the enhancement of rat 25K casein mRNA accumulation. It stimulates its formation and markedly increases the half-life of the 25K transcript. Those experiments were conducted in the presence of insulin, but this study shows that prolactin's effect on the half-life of the mRNA does not require insulin. However, prolactin exerts no effect on the transcription of the 25K casein mRNA in the absence of insulin. Both insulin and prolactin are essential for formation of the transcript. In other words, stimulation of transcription of the 25K casein gene cannot be ascribed to a single hormone.

Insulin has been reported to favor the accumulation of mRNA's for albumin (*2*), amylase (*3*), tyrosine aminotransferase (*4*), pyruvate kinase (*5*), and δ-crystallin (*6*). It has also been reported to inhibit the transcription of the phosphoenolpyruvate carboxykinase gene (*12*). However, the effect of insulin reported here and its role in the insulin-estrogen-ovalbumin system (*13*) suggest that this hormone may be an essential component in a number of transcription systems.

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Carbohydrate Dramatically Influences Immune Reactivity of Antisera to Viral Glycoprotein Antigens

Abstract. *Analysis of the ability of heteroantisera, monoclonal antibodies, and antibodies to synthetic peptides to react with viral glycoproteins deglycosylated with endoglycosidase F revealed that the reactivities of most of the antibodies to these glycoprotein antigens were influenced by the attached carbohydrate moieties. All heteroantisera prepared in rabbits or goats to either fully glycosylated retroviruses or influenza virus were virtually unreactive toward the viral glycoproteins after carbohydrate removal. Analyses with a panel of monoclonal antibodies to purified Rauscher murine leukemia virus gp70 indicated that the reactivity of most of these antibodies improved while the reactivity of others decreased or remain unchanged after carbohydrate removal. Most of the antibodies to synthetic peptide sequences in the influenza virus hemagglutinin also improved in reactivity after carbohydrate removal. These data indicate that carbohydrate side chains on viral glycoproteins influence the immune response to these antigens, and the more native the glycoprotein immunogen, the more dramatic the carbohydrate influence. Thus the immune response to these glycoproteins is not simply a function of the immunogenicity of certain domains over others but rather is a direct measure of carbohydrate influences on the host's perception of the foreign antigen.*

The pathways and mechanisms by which carbohydrate is added to glycoproteins are now relatively well defined (*1*). However, little is known about the role carbohydrates play in the function of this class of proteins. A glycosylated molecule may have increased hydrophilicity in certain of its regions or be protected from proteolytic attack; glycosylation may somehow facilitate the secretion of certain proteins or their mobilization to the cell surface (*2*). One of the primary concerns of our studies was to determine what role or roles carbohydrate may play in the immune response to viruses, in particular to retroviruses and influenza viruses. There has been considerable speculation on this subject, but until recently, reagents were not available that could effectively remove carbohydrate without proteolysis or modification of the primary amino acid backbone. The discovery of an endoglycosidase [Endo F (*3*)] that efficiently cleaves both *N*-linked high mannose and complex glycans from glycoproteins allowed us to test directly certain aspects of the carbohydrate-protein interaction. We now report that the carbohydrate moieties of the viral glycoproteins have a dramatic effect on the antigenicity of these glycoproteins.

Untreated and deglycosylated Rauscher leukemia virus (R-MuLV) were studied by Western blot analysis of heteroantisera prepared to purified R-MuLV glycoprotein (gp70), to intact R-MuLV, and to R-MuLV disrupted with a mixture of Tween and ether (Fig. 1). All three antisera showed diminished reactivity to gp70 after treatment of the virus with Endo F. The reactivity of the antiserum to purified gp70 (Fig. 1A) was only 40 percent of that of control after carbohydrate was removed from the viral glycoprotein (calculated by slicing and counting the isotopically labeled bands from the nitrocellulose strips). The reactivities of the antisera to intact virion (Fig. 1B) and to Tween-ether–disrupted virion (Fig. 1C) with deglycosylated gp70 were 1 to 3 percent of that observed with