# Turkey and Chicken Prolactins Stimulate the Proliferation of Rat Nb2 Lymphoma Cells

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*Abstract.* **The purpose of this investigation was to assess the actions of two avian prolactins (PRL) on rat Nb2 lymphoma cell proliferation. Various turkey and chicken PRL preparations stimulated the proliferation of rat Nb2 cells. The ability of avian PRL preparations to influence the behavior of rat Nb2 lymphoma cells provides a useful method for monitoring the PRL-like actions of native and recombinant avian PRL and suggests a potential role of PRL in modulating the avian immune system.**

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Prolactin (PRL) is an anterior pituitary hormone with a wide range of biological actions in vertebrates (1). In avian species, PRL have been implicated in the control of Columbid crop-sac development and brood patch development in some avian species (2, 3), and very recently in the control of lymphocyte proliferation in the chicken (4). The actions of PRL in avian species have largely been determined through study of the actions of purified mammalian PRL on avian tissues. Avian PRL have been purified (5–8), antibodies have been generated, and concentrations of circulating PRL have been measured (6, 9–11). Structural features of avian PRL have been further elucidated through the cloning of cDNA to chicken and turkey PRL (12–15). Isolation of chicken PRL cDNA has led to the generation of recombinant chicken PRL (16).

A major difficulty in studying avian PRL has been the availability of a method for monitoring their biological activities. In this article, we demonstrate that two avian PRL (turkey and chicken) effectively stimulate the proliferation of rat Nb2 lymphoma cells.

## Materials and Methods

**Hormones.** Porcine PRL (USDA-pPRL-B-1) and chicken growth hormone (AFP-7678B) were obtained from the National Hormone and Pituitary Program. The turkey PRL and turkey growth hormone preparations have been described previously (7, 17). Turkey luteinizing hormone (LH B221B) was provided by Dr. W. H. Burke, University of Georgia (18). Turkey follicle stimulating hormone (FSH) was purified from turkey pituitaries to produce a homogeneous preparation approximately three times the potency of porcine FSH (USDA-pFSH-B-1), as assessed by radioreceptor assay (J. A. Proudman, unpublished observation).

**Animals and Tissue Preparation.** White Leghorn chicken eggs were purchased from HyVac (Gowrie, IA). Eggs were incubated in a Humidaire incubator (Humidaire, New Madison, OH) at 38°C and 60% relative humidity. Pituitaries were dissected from newly hatched chicks. Turkey pituitaries were collected at a poultry processing plant from birds of random age and sex and stored at −70°C until use.

Cytosol preparations were generated by homogenizing the pituitaries in cold ammonium bicarbonate buffer (100 m$M$ $NH_4HCO_3$, pH 9.3, 100 m$M$ NaCl, 0.5 m$M$ phenylmethylsulfonyl fluoride) with a Brinkman Polytron tissue homogenizer (Brinkman Instruments, Westbury, NY). Homogenates were further disrupted by sonication for 1 min in a Microultrasonic Cell Disrupter (Kontes Co., Vineland, NJ). Following sonication, the homogenates were centrifuged at 12,000$g$ for 15 min. The pellet was discarded, and the supernatant centrifuged at 100,000$g$ for 60 min. The final supernatant was defined as the cytosol fraction. Protein concentrations of cytosol preparations were estimated by the method of Bradford (19).

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Plasma samples from laying and incubating turkey hens were collected from a Medium White strain (BUTA-8) raised in floor pens and trapnested five times per day. Birds were placed on a stimulatory photoperiod (14:10-hr light:dark cycle) at 28 weeks of age and samples were collected between 37 and 44 weeks of age. Birds were classified as incubating if they were found on the nest at least four times per day for at least four consecutive days without laying an egg and exhibited incubation behavior (ranging from reluctance to leave the next to aggressive defense of the nest). Birds classified as laying had laid between three and seven eggs during the preceding week. Blood was collected from incubating or laying turkeys, and plasma was isolated and stored at −70°C until tested in the rat Nb2 lymphoma cell proliferation assay or the turkey PRL radioimmunoassay.

**Rat Nb2 Lymphoma Cell Proliferation Assay.** The ability of various hormone preparations to stimulate the proliferation of rat Nb2 lymphoma cells was assessed. The Nb2 cell proliferation assay was conducted as described previously (20), with minor modifications. Cells were routinely grown in Fischer's medium supplemented with 50 $\mu M$ 2-mercaptoethanol, antibiotics (penicillin, 50 units/ml, and streptomycin, 50 $\mu$g/ml), 10% horse serum, and 10% fetal bovine serum in an atmosphere of 5% $CO_2$ and 95% air at 37°C in a humidified incubator. Twenty-four hours prior to initiating the assay, cells were diluted to a concentration of 100,000 cells/ml and incubated with Fisher's medium supplemented with 2-mercaptoethanol, antibiotics, and 10% horse serum. At the initiation of the assay, cells were washed, counted with a hemacytometer, and aliquoted to 16-mm wells (100,000 cells/well) of a 24-well culture plate. Hormone preparations were added to the wells at various concentrations (final incubation volume of 1 ml) and incubated for 72 hr. Samples of

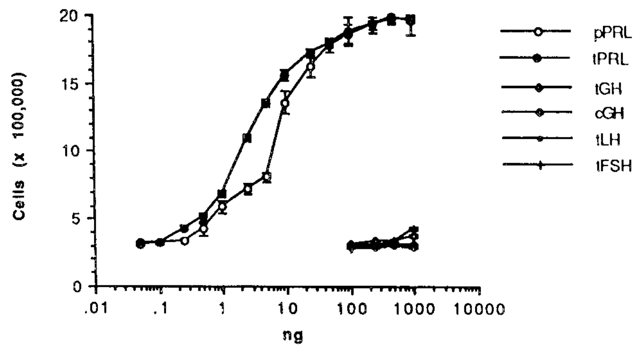


**Figure 1.** Dose-response curves for porcine PRL (pPRL) turkey PRL (tPRL), turkey luteinizing hormone (tLH), turkey follicle stimulating hormone (tFSH), turkey growth hormone (tGH), and chicken growth hormone (cGH) in the Nb2 rat lymphoma cell proliferation assay. Note the stimulatory actions of porcine and turkey PRL and the lack of response of the other hormones. Each point represents the mean of triplicate determinations and the vertical lines represent the standard error of the mean.

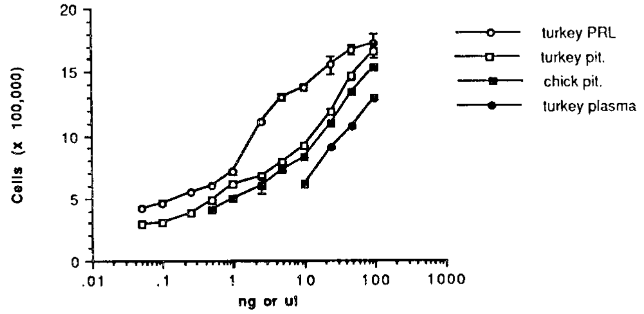


**Figure 2.** Dose-response curves for turkey PRL, turkey pituitary extracts, turkey plasma, and chicken pituitary extracts. Note the parallelism of the avian tissue preparations with the turkey PRL standard. The volumes of turkey plasma used in the assay have been multiplied by 10 in order to better visualize the dose-response relationship. Each point in the figure represents the mean of triplicate determinations and the vertical lines represent the standard error of the mean.

**Table I.** Serum Prolactin Levels in Laying and Incubating Turkey Hens Measured by Radioimmunoassay and Rat Nb2 Lymphoma Cell Proliferation Assay[a]

| Reproductive state | $n$ | Immunoassay (ng/ml) | Proliferation assay (ng/ml) |
|---|---|---|---|
| Laying | 4 | 136.3 ± 44.5 | 251.0 ± 55.2 |
| Incubating | 3 | 330.0 ± 33.2[b] | 726.7 ± 150.2[b] |

[a] Mean ± SE.
[b] Values for incubating hens are significantly different from values for laying hens. $P < 0.05$.

treated cells were collected and counted in a Sysmex Microcellcounter (model CC-110; TOA Medical Electronics, Japan). Each experiment was performed in triplicate and repeated at least three times.

**Turkey PRL Radioimmunoassay.** The measurement of plasma turkey PRL was performed with a homologous radioimmunoassay, as described previously (10).

## Results and Discussion

Avian PRL effectively stimulate the proliferation of rat Nb2 lymphoma cells *in vitro*. The response of Nb2 lymphoma cells to avian PRL was parallel with Nb2 lymphoma cell responses to porcine PRL (Fig. 1) and was specific for PRL (Fig. 1). Avian growth hormones, turkey luteinizing hormone, and turkey FSH failed to stimulate lymphoma cell proliferation (Fig. 1). The response was sensitive (range, 250–500 pg/well for both porcine and turkey PRL) and reproducible (intraassay and interassay variation were 3.5% and 6%, respectively) in agreement with the original description of the response of rat Nb2 lymphoma cells to mammalian PRL (20). PRL present in extracts of turkey and chicken anterior pituitaries and PRL present in turkey plasma generated parallel dose-response curves with the turkey PRL standard (Fig. 2). Concentrations of PRL in plasma from turkeys of different reproductive conditions measured with the rat Nb2 lymphoma cell proliferation assay showed excellent correlation with PRL measured by a specific turkey PRL radioimmunoassay (correlation coefficient, 0.94; Table I). The absolute concentrations of PRL detected with the Nb2 lymphoma cell proliferation assay were somewhat higher than concentrations measured with the PRL immunoassay, but both techniques demonstrated the significant increase in plasma PRL levels that is characteristic of the transition from egg laying to incubation in the turkey hen (for review, see Ref. 21). Similar discrepancies between serum PRL measurements using biological and immunological methods have been reported (22–24). The validity of using the rat Nb2 lymphoma cell proliferation assay for the precise monitoring of PRL concentrations in unpurified biological fluids is questionable.

The findings reported in this study have two important implications for research in the area of avian endocrinology. First, the rat Nb2 lymphoma cell proliferation assay, originally developed for the assessment of mammalian lactogenic hormones (20), is also a reliable tool for the rapid assessment of biological activities of avian PRL. This assay is far superior to the pigeon crop-sac assay in terms of its sensitivity and reproducibility (20). The lymphoma cell assay will prove to be extremely useful in comparing the biological activities of native and recombinant avian PRL. Second, our observations and those of Skwarlo-Sonta (4) indicate that avian PRL may have a role in modulating the immune system. The ability of PRL to modulate immunological function and to potentially control immunological responses to diseases has significant implications for the poultry industry.

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