

TABLE V. Oviducal Glycogen Content per Unit of DNA as Influenced by Estrogen Stimulation.

Oviduct section	Glycogen ($\mu\text{g}/100 \mu\text{g}$ of DNA)			
	Immature ^a	Immature + estradiol ^b	Laying hen	Nonlaying hen
Infundibulum	3.0	11.2	10.3	6.2
Magnum	2.7	6.3	6.2	3.7
Isthmus	2.8	8.6	29.2	4.1
Uterus	4.0	8.7	13.4	4.1

^a Twelve-week-old chick.

^b Twelve-week-old chick injected with 300 μg of 17 β -estradiol 6 hr before killing.

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Quantitative Analysis of Prolactin by Disc Electrophoresis and Its Relation to Biological Activity (33830)

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Up to the present time the pigeon crop sac response has been the most extensively used method for the assay of prolactin, and many modifications of this method have been reported (1, 2). Recently, disc electrophoresis was employed for semiquantitative analysis of prolactin in the anterior pituitary of several species (3-6). However, a quantitative method of analysis of prolactin by this method and its relation to the values obtained by the pigeon bioassay have not been investigated. The present experiments were carried out to study this problem. The possibility of using this method to measure prolactin in medium after incubation of anterior pituitary tissue was also examined.

Methods and Results. Disc electrophoresis on polyacrylamide gel was carried out by the method of Davis (7). The concentration of

acrylamide was 7.5% and a current of 4 mA/column was applied at pH 9.5. After electrophoresis, the column was stained in 1% amido schwarz solution for 1 hr. The optical density of prolactin band was measured with a microdensitometer (Canalco, model E).

Relation between dose of standard prolactin and optical density of the band obtained by disc electrophoresis. A standard bovine prolactin preparation (NIH-P-B2, 20 IU/mg)¹ was dissolved in distilled water, and electrophoresis was performed on 10-150 μg . Figure 1 shows the relationship between the dose of prolactin and the optical density of the bands obtained. The regression equa-

¹The standard prolactin preparation was kindly provided by the Endocrinology Study Section, NIH.

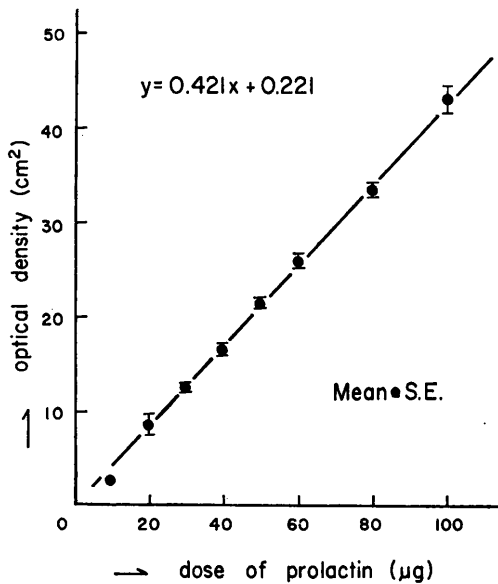


FIG. 1. Standard curve for microdensitometric assay of prolactin (NIH-P-B2) separated by disc electrophoresis.

tion which described this relationship for 10–100 µg of standard prolactin was $Y = 0.421X + 0.221$, where Y was the optical density and X was the dose of standard prolactin. The slope was significantly different from zero ($p < 0.01$) and the equation contained no significant deviation from linearity.

Pituitary prolactin content of rat injected with bovine prolactin. In an attempt to prove that the fastest migrating band was prolactin in the electrophoresis of anterior pituitary tissue from rats, the optical density and the bioassay values of the anterior pituitary of rats chronically treated with prolactin were compared with those of untreated rats. Female rats of the Wistar strain, about 10 weeks old and weighing 200–250 g each, were

subcutaneously injected with 5.0 mg of standard bovine prolactin¹ dissolved in 0.4 ml of distilled water, twice daily for 7 days and once in the morning of the eighth day. They were killed 7 hr after the last injection. Similar untreated rats served as controls and were killed at the diestrous stage of the estrous cycle. The anterior pituitaries were rapidly removed and weighed. About $\frac{4}{5}$ of each pituitary was weighed, homogenized in 0.1 ml of distilled water and used for disc electrophoresis. The remaining $\frac{1}{5}$ of the pituitary was suspended in 0.5 ml of distilled water, and used for measuring prolactin activity by the sensitive pigeon crop sac method of Nicoll (2). The paired comparison method was employed, by injecting the materials from the experimental and control rats over each side of the same crop sac of 6–8-week-old white king squabs. The results are presented in Table I. The optical density of the prolactin band from the experimental rats was decreased to 59% of the controls. This proves that the specific band was prolactin itself. The pigeon crop sac assays showed a similar relationship between the treated and control groups. These results indicate that a good correlation exists between the optical density of the electrophoretic band and the biological activity of prolactin.

Correlation between optical density of prolactin band and its biological activity. Rat pituitary tissue in amounts of 2 or 20 mg were homogenized in 0.2 ml of water, and equal volumes of each were placed on two columns of polyacrylamide gel for electrophoresis. After electrophoresis, one column was stained and the optical density of the prolactin band was measured; the other pro-

TABLE I. Pituitary Prolactin Content of Rats Injected with Bovine Prolactin.*

Group	No. of rats	Body wt (g)	AP wt (mg)	AP prolactin			
				Electrophoretic assay (OD: cm ²)		Bioassay (MDW: mg)	
				(/whole pit.)	(/mg of pit.)	(/whole pit.)	(/mg of pit.)
Control	6	226 ± 11	6.3 ± 0.3	7.1 ± 1.0	1.1 ± 0.1	52.9 ± 13.5	8.96 ± 2.70
Exptl.	6	234 ± 6	6.5 ± 0.2	4.2 ± 0.8	0.7 ± 0.1	38.5 ± 6.5	6.06 ± 1.10

* Values = mean ± SE; AP = anterior pituitary; MDW = mucosal dry weight.

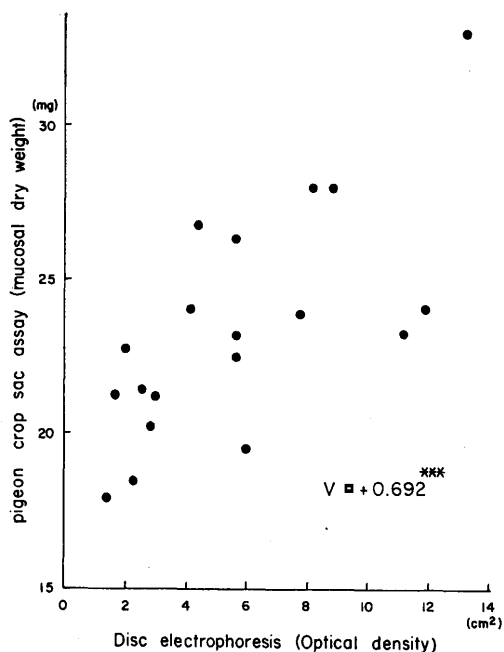


FIG. 2. Correlation between optical density of PL band and its biological activity.

lactin band was cut and removed without being stained and was homogenized in 2.5 ml of water. Prolactin was eluted by the method of Kragt and Meites (8) and assayed in six squabs per gel. The results are shown in Fig. 2. The correlation between the optical density of the prolactin band and its biological activity was highly significant ($r = +0.692$, $p < 0.01$). This indicates that the optical density of the prolactin band gives a good estimate of its biological activity.

Determination of prolactin released from pituitary of rat. Single rat pituitary was cut into 4 quarters and incubated in medium 199 (Difco Labs., Detroit, Michigan) by the method of Kragt and Meites (9). After incubation the medium was filtrated on Sephadex G-25 to exclude the low molecular substances which might interfere with the electrophoresis. Aggregation of prolactin on Sephadex column could be prevented by the addition of urea (gm) and EDTA (1 mM) to the medium as described by Saxena and Heneman (10). The effluent was lyophilized and dissolved in 0.15 ml of water and tested by disc electrophoresis. Figure 3 shows the elec-

trophoretic patterns of the medium after incubation. The sharp single band was found at the same site as the prolactin band of pituitary homogenate after electrophoresis.

The prolactin released by anterior pituitary from lactating rats after litter removal was also studied by this method. Primiparous lactating rats of the Sprague-Dawley strain were used. After postpartum, each litter was adjusted to 12 pups on postpartum day 1. On postpartum day 10, the pups were removed from the mother, and 0, 8, or 16 hr later the mothers were killed by decapitation. The anterior pituitary was rapidly removed, cut into 4 quarters and incubated for 4 hrs. After incubation, the medium was filtrated and lyophilized as described previously. The prolactin released from the pituitary of rats 5 days after weaning on postpartum day 25 was also

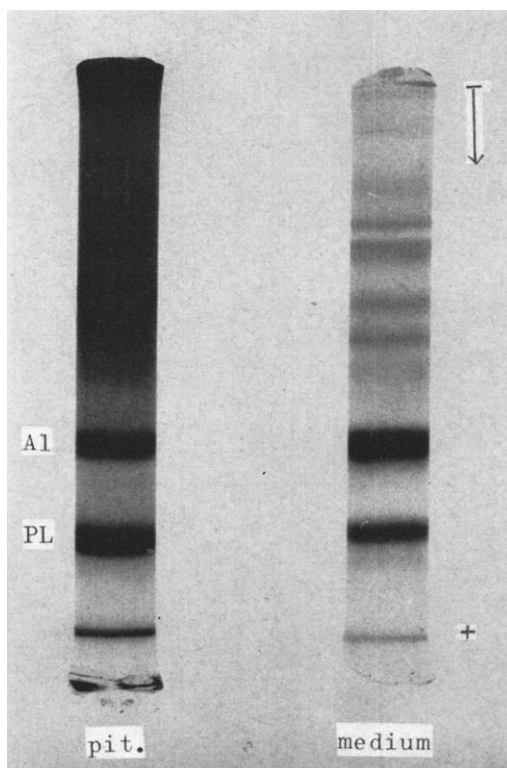


FIG. 3. Electrophoretic pattern of incubation medium of pituitary gland: Al = albumin; PL = prolactin; pit. = normal pituitary of mature female rat; medium = incubation medium of pituitary of female rat.

TABLE II. Prolactin Released *in Vitro* from Anterior Pituitary of Lactating Rats after Litter Removal.^a

Duration after litter removal (hr)	No. of rats	Body wt (g)	Pituitary wt (mg)		Ov. wt (mg)	Ad. wt (mg)	Prolactin released (OD: cm ²)	
			WW	DW			(/whole pit.)	(/mg pit.)
0	6	334 ± 14	16.6 ± 1.0	2.52 ± 0.21	89.4 ± 5.6	94.6 ± 4.4	3.7 ± 1.0 ^b	0.22 ± 0.05 ^f
8	6	345 ± 4	16.6 ± 1.4	2.50 ± 0.13	89.1 ± 3.5	97.2 ± 6.9	8.8 ± 0.8 ^d	0.56 ± 0.03 ^g
16	6	345 ± 9	15.0 ± 1.3	2.34 ± 0.20	83.4 ± 5.6	82.4 ± 4.5	5.4 ± 0.7 ^e	0.38 ± 0.06 ^h
5 days after weaning	6	307 ± 10	15.1 ± 0.8	2.23 ± 0.16	101.6 ± 1.6	76.0 ± 3.7	3.1 ± 0.9	0.21 ± 0.06

^a Values = mean ± SE; WW = wet weight; DW = dry weight.

^b c-d, f-g: $p < 0.01$. d-e, g-h: $p < 0.05$.

examined. The results are presented in Table II. Prolactin released from the pituitary after 8 hr of nonsuckling was significantly greater than after 0 or 16 hr of nonsuckling ($p < 0.01$ or 0.05). The anterior pituitary of the mother rat after 16 hr of nonsuckling released more prolactin than after 0 hr, although the difference was not statistically significant. The pituitary of rats 5 days after weaning released almost the same amount of prolactin as that of rats after 0 hr of nonsuckling. Pituitary weight was not significantly different among groups.

Discussion. The linear relationship between the dose of standard NIH bovine prolactin and the optical density of the band obtained by disc electrophoresis indicates that this method can be employed for quantitative assay of prolactin. The minimum detectable dose was 10 μ g of the standard prolactin preparation, which is equivalent to 200 mU. This method can clearly detect the prolactin in 1–2 mg of anterior pituitary from mature female rats.

Identification of the prolactin band of the anterior pituitary of rats has been reported by several investigators (5, 8, 11), by assaying the biological activity of eluates of the bands. The present study shows that exogenous administration of prolactin reduced not only the optical density of the prolactin band but also its biological activity, further confirming that this band is prolactin. Recently it was reported that chronic administration of prolactin (12) or transplantation of mammotropic pituitary tumors (6, 13, 14) decreased pituitary prolactin content. Also, implants of prolactin into the median eminence of rats reduced pituitary prolactin concentration (15). The present results are in good agreement with these reports.

Analysis of prolactin in the medium after incubation by disc electrophoresis has not been reported except for the study of Takizawa *et al.* (16), who dialyzed the incubation medium. We found that filtration and lyophilization of the medium was more suitable than dialysis for 4 hr. One pituitary of a mature female rat or two pituitaries of a

mature male rat was sufficient to detect the prolactin released into the medium.

It was observed that the anterior pituitary of postpartum rats after 8 and 16 hr of nonsuckling released about 2.4 and 1.5 times as much prolactin, respectively, as that of normal lactating rats (0 hr of nonsuckling) on postpartum day 10. Grosvenor *et al.* (17) found that 0.5 hr of suckling after 8 hr of nonsuckling decreased pituitary prolactin concentration in the lactating rat, but there was no fall if the period of nonsuckling was extended to 16 hr even though the pups obtained normal quantities of milk. According to their report, the pituitary prolactin content after 8 hr of nonsuckling was only 14% more than that after 16 hr of nonsuckling. In the present experiment, prolactin released after 8 hr of nonsuckling was 63% more than that after 16 hr of nonsuckling. Therefore, the difference in prolactin released between 8 and 16 hr of nonsuckling was not due only to the initial pituitary content of prolactin before incubation. Pasteels (18) noted that rat pituitary prolactin cells were engorged with granules after 10 hr of nonsuckling. It is probable that the pituitary after 16 hr of nonsuckling shows a lower capacity to secrete prolactin. Further investigation of this problem is now in progress.

Summary. The quantitative analysis of prolactin was investigated by disc electrophoresis. A linear relationship was found between the amount of standard prolactin preparation used and the optical density of the band obtained, indicating that this method can be employed for the quantitative determination of prolactin. One–2 mg of fresh pituitary from mature female rats was sufficient for clear detection of prolactin. A high correlation was obtained between the optical density and the biological activity of the eluate of the band. Chronic administration of large doses of prolactin to rats decreased the optical density of the prolactin band to 59%

of that in the control pituitary. It was also demonstrated that prolactin released *in vitro* from the anterior pituitary of rats could be detected by disc electrophoresis.

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