Prolactin Production by Rat Anterior Pituitaries Cultured *in vitro* as Estimated by Crop Gland Assay, Densitometric Analysis and Radioimmunoassay[1] (36360)

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Adequate evidence is now available to indicate that when the mammalian anterior pituitary (AP) is cultured *in vitro* there is a marked increase in the synthesis and release of prolactin (1). Factors important for maximum prolactin production in the short-term culture (3 days) of the rat AP have recently been reported (2), however, extended culture under these conditions resulted in the cessation of prolactin production after 8 or 9 days (Gala, unpublished observations). Prolactin activity of AP tissue and of culture medium has been classically estimated using the pigeon crop gland assay (3, 4). In recent years newer methods have been introduced for the estimation of prolactin. The separation of prolactin by analytical disc electrophoresis and its quantification by microdensitometry was reported to be less expensive and more precise than the crop gland assays (5). The availability of purified rat prolactin allowed the development of radioimmunoassays for the detection of prolactin (6–8). In this study rat AP were cultured in an air environment and the prolactin activity of the medium was estimated by the intradermal crop gland assay, by disc electrophoresis and microdensitometry (ED) and by radioimmunoassay (RIA).

Materials and Methods. Anterior pituitaries were aseptically removed from 40 sexually mature female Sprague–Dawley rats 200 g in body weight and cultured in Medium 199 containing 200 U/ml each of penicillin and streptomycin, and 50 U/ml of Mycostatin. Ten AP were cultured in a single 60-mm dish containing 8.0 ml of medium; a total of 4 dishes were used. The culture was performed in an air environment for a period of 54 days and the medium was changed every 3 days. Medium from each change was pooled for subsequent assay. Details of the culture technique as well as the method used to estimate the percentage of viable tissue have been presented elsewhere (2).

Prolactin activity of the medium was bioassayed using the ratio method of the intradermal crop gland assay (2). With this method a standard amount of prolactin (3 μg of NIH-P-S-7, 24.3 IU/mg) was injected on one side of the crop gland while the medium was injected on the other side. A ratio of the degree of proliferation of standard to unknown was calculated and the prolactin concentration estimated from a standard curve. A total of 6–9 birds were used for each medium change.

In the ED analysis varying amounts of rat prolactin (NIH-NIAMD-RP-1), ovine prolactin (NIH-P-S-7) and human growth hormone (NIH-GH-HS-1207A) were dissolved in Medium 199 and separated by disc electrophoresis using a 2.5% spacer gel, a 6.0% separating gel and a Tris–glycine buffer of pH 8.9. The gels were stained with 0.5% amido black in 7% acetic acid for 1 hr and destained electrophoretically. The staining intensity of the bands was determined using the Gelscan microdensitometer (Gelman Instrument Co.). The standard curves are

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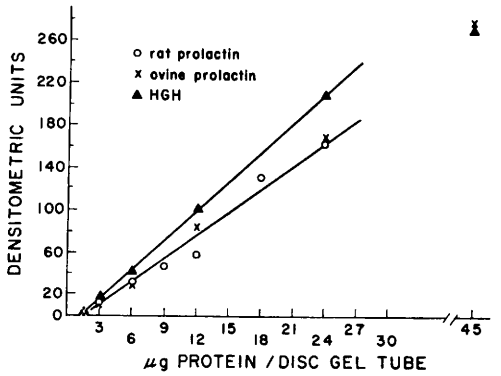

FIG. 1. Standard curves for densitometric analysis of analytical disc electrophoresis using the Gelscan microdensitometer (Gelman Instrument Co.). A single line was drawn through the ovine and rat prolactin values.

presented in Fig. 1. Rat and ovine prolactin gave similar results and were presented as a single curve; the slope of the HGH curve, however, was somewhat different than that of the prolactins. Three separate disc electrophoreses containing 5 medium samples and a 9-μg rat prolactin standard in duplicate were performed to determine the prolactin content of the medium. In some instances the same medium was run in two separate experiments. The sample volume was 300 μl for both medium and rat prolactin standard. The densitometric reading of the prolactin standard in each electrophoresis was used to calculate the protein content of the medium in that electrophoresis. Each point on the curve in Fig. 2 represents 2–4 observations that agreed with each other within 10%.

The RIA of the prolactin content of medium was determined using an antiserum generated to lyophilized rat AP culture medium and was absorbed with hypophysectomized rat serum. NIAMD rat prolactin was used for iodination and standards. The method has recently been presented (9) and will be published in greater detail elsewhere. Medium samples for RIA were diluted 1:4,000, 1:10,000, and 1:20,000, and duplicate tubes were run for each dilution. Each point in Fig. 2 for the RIA curve represents 3 observations which agreed with each other within 20%.

Results. The culturing of rat AP in an air environment resulted in prolactin production for a period of 33 days (Fig. 2). Medium examined after this period did not contain detectable prolactin. Histologic examination vealed that only 1–2% of the tissue was viable at the end of 54 days of culture. The amount of prolactin initially introduced into the culture system with the AP explant was approximately 0.52 μg/mg as estimated by crop gland bioassay. Using this value and 5 mg AP tissue/ml of medium it was calculated from the bioassay data that the net synthesis of prolactin for the 33 days of culture was approximately 69-fold or 2.1-fold per day.

The results of prolactin assays from the three methods were similar in that there was a decrease in activity for the first few medium changes with an increase thereafter to the 33-day medium change, followed by a precipitous drop to undetectable levels from the 36th day onward (Fig. 2). Further examination of the curves revealed that the ED and RIA systems were more closely related to each other with the lowest prolactin activity observed on the 15th day of culture followed by a gradual rise to the 27th day. Bioassay did not detect a distinct low point but a low level of prolactin activity was observed between 12 and 24 days of culture. The peak of prolactin activity on the 33rd day of culture detected by the ED method was not recorded by either the bioassay or RIA methods. If bioassay were used as a basis for compari-

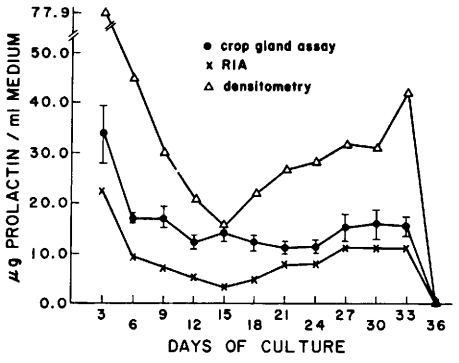

FIG. 2. The long term culture of rat anterior pituitaries (AP) in an air environment. Vertical lines for crop gland assay represent the standard error of the mean.

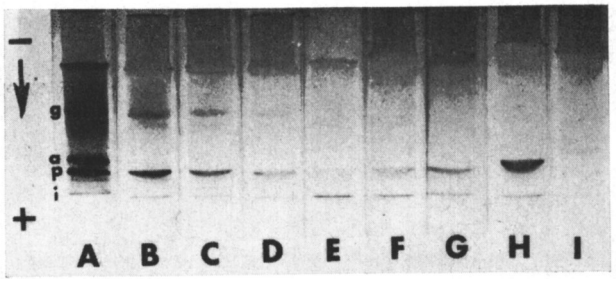

FIG. 3. Analytical disc electrophoresis of AP culture medium. A 300-μl sample was layered onto 2.5% spacer gel and separated in a 6.0% gel using a Tris–glycine buffer with a pH of 8.9. Gels A–I represent 3, 6, 9, 12, 15, 18, 21, 33, and 36-day medium changes. i = ion front, p = prolactin, a = albumin, g = growth hormone.

son, then the ED method resulted in overestimating the amount of prolactin by a factor of 2 to 3, while RIA resulted in underestimating the prolactin present by a somewhat lesser magnitude. The ED method gave values 3–4 times higher than the RIA.

Analytical disc electrophoresis of the medium revealed three major components for the first medium change (Fig. 3A). The albumin component disappeared after the initial medium change while the growth hormone component continued to be visible up to the 12th day of culture. From the 18th to the 33rd day of culture the prolactin component increased in the medium while the growth hormone component was not observed (Fig. 3, F–H). On the 36th day of culture and thereafter (Fig. 3, I) there appeared 3 to 4 new components; these proteins were probably the products of AP tissue necrosis.

Discussion. Whenever a new prolactin assay system was introduced, it had been compared with the crop gland bioassay for validation. A number of investigators using varied experimental conditions have reported a good correlation between bioassay and ED systems for estimating prolactin activity (5, 10, 11). In most cases, however, the correlation had been drawn on a relative basis rather than by converting to similar units. Nicoll *et al.* (5) observed that 1 μg of rat prolactin estimated by bioassay gave 3.3 μg when estimated by ED system although relative changes between the two systems were in good agreement. The data in Fig. 2 was in general harmony with the relative correlation between bioassay and ED observed by others, but as with the observations of Nicoll *et al.* (5) there was an overestimation of prolactin when determined by ED method.

The results of rat prolactin RIA and bioassay have also been found to have good correlation when considered on a relative basis (7, 8). On an absolute basis, some investigators observed an underestimation of prolactin activity of some preparations by RIA (7), while others noticed no difference (8) when compared with bioassay. Ovine and bovine prolactin RIA systems have been reported to overestimate (12) or underestimate (13) prolactin content in comparison with bioassay. Our RIA system gave prolactin values lower than those obtained by bioassay, and on a relative basis followed the ED data better than the bioassay data. The better correlation between RIA and ED systems may reflect the greater precision of these methods over that of the crop gland. To our knowledge a comparison between the rat RIA system and the ED system for estimating prolactin content has not been reported before.

It has been observed previously that rat AP cultured in a 95% O_2–5% Co_2 environment produce 2–3 times more prolactin per unit of time than when cultured in an air environment (14, 15). A 95% O_2–5% CO_2 environment was, however, not conducive to prolactin production or AP tissue survival in long term culture (Gala, unpublished observations). In an air environment the rat AP continued to produce prolactin for 33 days in a culture system that did not induce cell proliferation. This represents a longer period of prolactin production *in vitro* than that

reported previously for rat AP organ cultures (14). The reason for the decrease in prolactin production in the initial few medium changes followed by an increase in the later days of culture is not apparent at this time. Whether the disappearance of GH from the culture system, as evidenced by disc electrophoresis, was related to the biphasic production of prolactin is not known. It is interesting to note that the prolactin activity as estimated by RIA and ED, began to increase shortly after the disappearance of GH in the medium (Fig. 3). In examining rat AP tissue cultures (16) and AP tumors (17), it had been reported that both prolactin and growth hormone were produced together. Our observation that growth hormone production was lost with extended culture in our system has important implications when one considers an approach to obtain primate prolactin free from growth hormone.

Summary. Anterior pituitaries (AP) were aseptically removed from sexually mature female Sprague Dawley rats and cultured in an air environment for a period of 54 days with a medium change every 3 days. The prolactin activity in the medium was determined by the intradermal crop gland bioassay, by disc electrophoresis–microdensitometric analysis (ED) and by radioimmunoassay (RIA). Prolactin activity was detected in the medium by all three methods up to the 33rd day of culture. There was a decrease in medium prolactin content up to the 15th day and an increase thereafter. Examination of the medium by disc electrophoresis revealed the loss of the growth hormone component beginning on the 15th day medium change. The three methods used to estimate prolactin activity produced results which were in general agreement on a relative basis. The ED and RIA methods, however, were more closely related to each other than to the crop gland assay. On an absolute basis (μg–μg) the ED method overestimated the prolactin content by a factor of 2–3 while the RIA underestimated prolactin content by 1 or less order of magnitude when compared to bioassay values. The reason for these differences was not apparent.

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