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ISOLATION OF PROLACTIN BY ACRYLAMIDE GEL ELECTROPHORESIS OF THE RAT PITUITARY TISSUE CULTURE MEDIUM

BY

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ABSTRACT

Biological, chemical, or immunological properties of prolactin are not yet completely understood. The relationship between pituitary prolactin and growth hormone or prolactin-like substance in the fluids such as blood and urine in human is another important problem to study. In order to understand these problems, pure prolactin is urgently required. In the present experiment isolation of prolactin from the rat pituitary tissue culture medium was attempted by acrylamide gel electrophoresis, utilizing the fact that the pituitary separated from hypothalamic connection secreted almost exclusively prolactin and that pituitary prolactin increased by estrogen administration. As a result of 7-day culture prolactin band was obtained separately. Presence of growth hormone band even after 7-day culture indicated existence of GIF (growth hormone-inhibiting factor) in the hypothalamus.

INTRODUCTION

Recently a radioimmunoassay has been applied for the measurement of prolactin. This method shows a greater sensitivity and a high degree of precision as compared to bioassay methods, but this method is still far from satisfaction, because pure prolactin for production of antiserum is not available. Although a biological, a chemical, or an immunological approach has been attempted to study the relationship between prolactin and growth hormone, no definite conclusion has been established. Two distinct substances were identified in animals such as sheep or pigs, but in human it is uncertain whether prolactin and growth hormone are properties of the same molecule.

The present paper describes the isolation of prolactin from the rat pituitary tissue culture medium by acrylamide gel electrophoresis.

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Materials and Methods

Animals. Female Wistar rats weighing 150 to 180 g were used. The animals were maintained at 25°±1°C in a light-controlled room (light from 800 to 2000 daily). Prior to the experiment the animal was injected with 25 μg of estradiol benzoate per day for 2 weeks; unless mentioned otherwise.

Pituitary tissue. The anterior pituitary was aseptically removed from the rat under ether anesthesia and sliced into 10 to 12 pieces. At the conclusion of culture the pituitary tissue was sectioned at 5 μ and stained with hematoxylin and eosin, azan, or periodic acid Schiff (PAS) method.

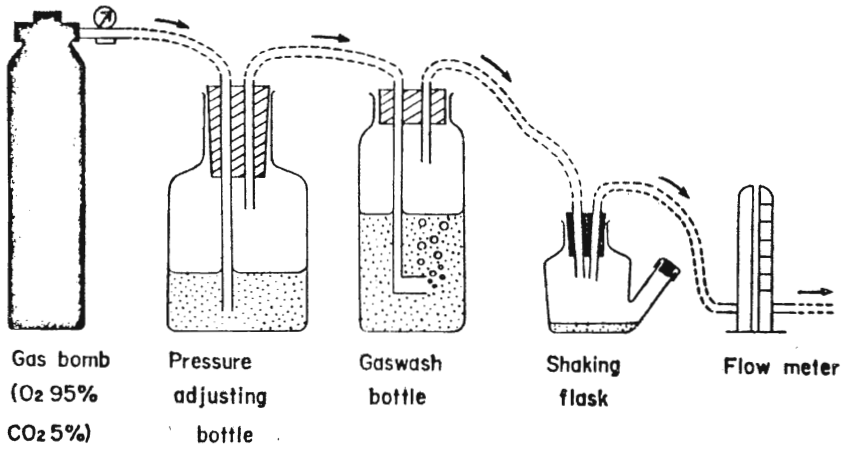

Fig. 1. Arrangement of gas line.

Organ culture. Sliced tissue of 20 pituitaries was distributed in a flask containing 3 ml of the tissue culture medium 199. No addition of serum or chick embryo extract was made. Two flasks were connected, as shown in Fig. 1, and fixed on a shaking incubater (60 rpm). The culture was done for 7 days at 37°C under a gas phase of 95% O_2 : 5% CO_2. The medium was renewed every day and each medium was frozen at −20°C.

Disc electrophoresis. Acrylamide gel electrophoresis was made in 7.5% gel at pH 9.5, according to the method of Ornstein[1].

Bioassay. The micro-method of pigeon crop sac gland described by Fujii et al.[2] was used for the measurement of prolactin. For growth hormone the tibia test by Greenspan et al.[3] was applied using Long Evans Rat.

Results

1. Histology of cultured tissue and prolactin content in the medium collected on the 7th day of culture: Forty anterior pituitaries from estrogen-

treated rats were cultured for 7 days. Histological appearance at the conclusion of the culture was almost similar to that of fresh pituitary except for a slight pyknosis. Prolactin content in the medium on the 7th day was 22.5 IU/ml (40 pituitaries).

2. Disc electrophoresis of fresh pituitary homogenate and 4-hr tissue culture medium: Anterior pituitaries from 40 estrogen-treated and 40 intact rats were respectively homogenized and centrifuged at 3000 rpm for 10 min. Supernatant and precipitate were lyophilized and examined. In disc electrophoresis, both supernatant and precipitate gave 3 major bands. Fig. 2 shows a pattern of the supernatant; band 1 and 2 were stained with the same degree in either groups, but band 3 was heavier in estrogen-treated rat than in intact rat. Fig. 3 shows patterns of precipitate, supernatant and medium collected at 4 hr. In the precipitate, band 2 was lighter; in the supernatant, band 3 was lighter; and in the medium, band 3 was lighter like the supernatant pattern.

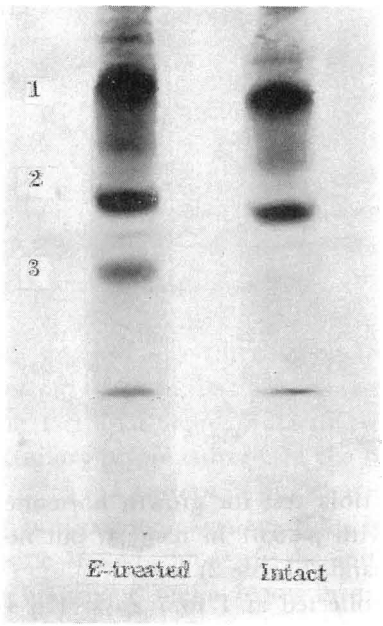

Fig. 2

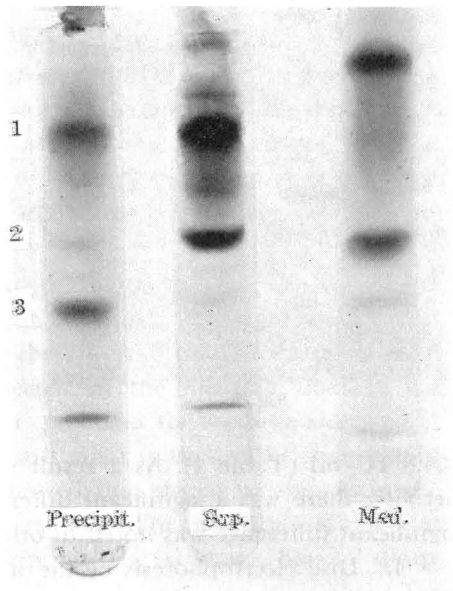

Fig. 3

3. Prolactin and growth hormone activities of the 3 bands: The media collected at 1–7 days gave 3 major bands in disc electrophoresis. Each band was homogenized with 0.09% NaCl solution and centrifuged at 3000 rpm for 10 min. The supernatant was bioassayed. Prolactin activity of band 1 was 1.2< <2.4 IU/ml, band 2 was negative, and that of band 3 was 2.4<

Table 1. Prolactin activity of the 3 major hands

Group	Prolactin content IU/ml
Band 1	1.2< <2.4
Band 2	<1.2
Band 3	2.4< <4.8
Saline	<1.2

Table 2. Growth hormone activity of the 3 major bands

Group	No. of rats	Tibial cartilage width[1]
Band 1	5	216±14[2]
Band 2	3	135±5
Band 3	3	143±16
Saline	3	139±11

1. $\mu \pm$S.E. 2. $p<0.01$

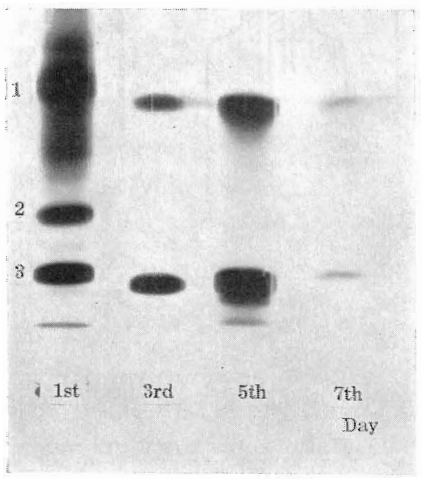

Fig. 4

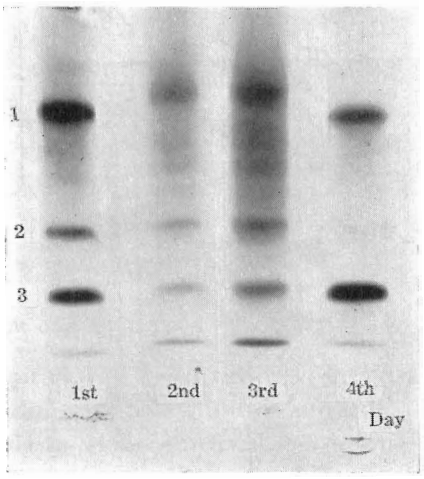

Fig. 5

<4.8 IU/ml (Table 1). As a result of the tibia test for growth hormone activity, there was a significant difference with $p<0.01$ in band 1, but no significant difference was noted in other 2 bands (Table 2).

4. Disc electrophoresis of the media collected at 1 to 7 days: Fig. 4 shows that the medium collected on the 1st day gave 3 major bands, but band 2 (supposed to be albumin) disappeared after the 3rd day. Band 1 and 3 were apparently noted till the 7th day (Fig. 4).

5. Disc electrophoresis of the media added with hypothalamic homogenate: Hypothalamic including median eminence from 28 rats were homogenized with 1.5 ml of the tissue culture medium 199. The pituitary alone was cultured on the 1st and 4th days, but on the 2nd and 3rd days 0.75 ml

of hypothalamic homogenate was added to the media, respectively. Fig. 5 shows disc electrophoresis of each medium. The intensity of band 3 was reduced as compared to band 1 on the 2nd and 3rd days. On the 4th day, without hypothalamic homogenate, band 3 was heavier than band 1.

Discussion

In the present experiment a histological appearance indicated that the pituitary tissue after 7-day culture maintained a secretive function. Prolactin content in the medium collected on the 7th day was 22.5 IU/ml (40 pituitaries). Both supernatant and precipitate of fresh pituitary homogenate gave 3 major bands in disc electrophoresis. Media collected at 4 hrs and on the 1st day also gave 3 major bands. Biologically distinct prolactin activity was noted in band 3, growth hormone activity, in band 1; and both activities were negative in band 2 (supposed to be albumin). It is able to conclude that band 1 and band 3 are biologically different substances. Furth and Moy[4] also showed 3 major bands in disc electrophoresis of extracts of normal female W/Fu rat pituitary or of pituitary from a rat bearing W_{15} tumor.

Previously Nicoll and Meites (*in vitro*)[5] and Okamoto (*in vivo*)[6] have demonstrated that estrogen increased prolactin content in the pituitary of the rat. As shown in Fig. 2, band 3 was heavier in estrogen-treated rat than in non-treated rat, although intensity of other 2 bands was similar. This electrophoretic pattern also indicated an increased pituitary prolactin by estrogen treatment.

A question arises whether prolactin contained in the culture medium was released from the tissue or leaked out. Meites et al.[7] demonstrated that not only leakage but also release from the cultured tissue took place from the fact that more prolactin was detected in the medium than in the pituitary before culture. In the present experiment the medium collected at 4 hrs gave a pattern in disc electrophoresis similar to that of the supernatant of pituitary homogenate. This result showed that stored substances in the tissue leaked out at the beginning of culture. The medium collected at 24 hrs gave 3 major bands in disc electrophoresis. Although band 2 disappeared after 3 culture days, bands 1 and 3 clearly appeared throughout the 7 days. This was an evidence that a secretive function was maintained in the pituitary tissue cultured for 7 days. As previously mentioned, growth hormone activity was noted in band 1 and prolactin activity was remarkable in band 3. Presence of PIF (prolactin inhibiting factor) in the hypothalamus has been demonstrated by many investigators[8–13] showing that the pituitary separated from hypothalamic connection secreted almost exclusively pro-

lactin. The present experiment indicated the possible presence of GIF (growth hormone inhibiting factor) in the hypothalmus, because band 1 appeared with band 3 throughout the 7 days. According to Deuben and Meites[14], growth hormone was released in the first 6 days from the rat pituitary cultured for 18 days. Recently, Krulich and McCann[15] also stated the possibility of GIF contained in the hypothalamus obtaining two zones by column chromatography of hypothalamic extract on Sephadex 25. One of these two zones promoted growth hormone release, while the other zone inhibited it. In the present experiment addition of hypothalamic homogenate to the culture medium resulted in heavier band 1 than band 3. However, without the hypothalamic homogenate, band 3 was heavier than band 1. There is a possibility that both GRF (growth hormone releasing factor) and GIF are contained in the hypothalamus. Krulich et al.[16] stated that normally GRF, which is superior to GIF in quantity, promoted growth hormone secretion from the pituitary.

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