RAPID COMMUNICATION

Synthesis of Two Lactogenic Proteins by
the Mouse Placenta in vitro

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Synopsis

The culture medium of mouse placental tissues was analyzed on acrylamide gel electrophoresis to localize lactogenic substances. Placental explants from 12- or 14-day-pregnant BALB/cHe mice were organ-cultured for 6 days in leucine-free Waymouth's medium supplemented with 3H-leucine (10 μCi/ml) and insulin (0.12 I.U./ml). The medium was collected every other day and subjected to acrylamide gel electrophoresis. The electrophoretic pattern of radioactive leucine incorporated into proteins was examined on stained 7% gels. Five protein bands were associated with high radioactivity. The location of lactogen activity on acrylamide gel was then investigated by the technique of organ culture of mouse mammary tissues. Placental explants from 12- or 14-day-pregnant BALB/cHe mice were organ-cultured in Waymouth's medium supplemented with insulin for 2 days. After electrophoresis, proteins were eluted by keeping the segment of acrylamide gel in phosphate buffer, dialyzed and dissolved in tissue culture medium 199 supplemented with insulin and cortisol. Mammary tissues from 8-day-pregnant KA2 mice were cultured for 3 days in the medium containing each eluate. Mammary glands always responded to eluted proteins from two positions of 7% gel, as judged in histological sections. The data suggest the presence of two lactogenic substances in the mouse placenta.

Secretion of mammotrophic substance by mouse chorionic cell was demonstrated by Kohmoto & Bern (1970) using organ co-culture of mouse mammary tissues with placental explants taken from day 6 to the end of pregnancy. The presence of large amounts of placental lactogen in the serum of pregnant women has been reported by many authors (Beck et al., 1965; Kaplan & Grumbach, 1965; Samaan et al., 1966; Spellacy et al., 1966; Saxena et al., 1969; Geiger et al., 1971; Spona & Janisch, 1971; Sato, 1973). Shiu et al. (1973) estimated the serum level of rat placental lactogen to be as high as 1584 ng/ml, using a radio-receptor assay. The role of this hormone remains uncertain both in man and in rodents. Isolation of mouse placental lactogen will permit determination of its levels in body fluids and help clarify its role in normal mammary development and tumorogenesis in this species. The present study presents initial data on the location of lactogenic proteins from the mouse placenta on polyacrylamide gel disc electrophoresis.

Placental tissues including cytotrophoblast and labyrinth area from 12- or 14-day-pregnant, nulliparous BALB/cHe mice were organ-cultured in leucine-free Waymouth's medium (MB 752/1) supplemented with 10 μCi/ml 3H-leucine and 0.12 I.U./ml bovine insulin (Novo, Denmark) for 6 days without addition of serum. Eight explants, each weighing approximately 2 mg, were cultured in 1 ml of the medium. Media were changed every other day and stored separately in a freezer until they were used.

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0.4 ml of each medium was subjected to analytical disc electrophoresis (7% polyacrylamide gel, pH 9.5). Gels were fixed in 5% trichloracetic acid (TCA) and stained with 0.01% amidoblock 10B. After rinsing out the unincorporated 3H-leucine by changing TCA daily for more than three days, gels were cut into 2-mm segments which in turn were oxidized in 0.1 ml 30% hydrogen peroxide at 60°C, then dissolved in 1 ml Hyamine 10 X at 60°C. Radioactivity was counted in a toluene-based scintillation mixture using an Aloka liquid scintillation counter 651 (Aloka, Tokyo) with a counting efficiency of 25-29%. Two explants were homogenized in 0.4 ml of 0.04 M phosphate buffer (pH 7.2). The whole homogenate was subjected to disc electrophoresis. Distribution of radioactivity of explants in gel was determined in the same way.

The location of proteins with lactogenic activity on 7% acrylamide gels was accomplished by assaying on organ cultures of mouse mammary tissues. Placental explants from BALB/cHe mice on day 12 or 14 of pregnancy were organ-cultured for 2 days in Waymouth's medium (MB 752/1) supplemented with 0.12 I.U./ml bovine insulin. Each culture contained 8 to 10 explants. Culture media were collected and subjected to analytical disc electrophoresis (7% acrylamide, pH 9.5). Gels were cut into 2-mm segments which were then allowed to stand for 2 days in 2 ml of 0.04 M phosphate buffer (pH 7.2) to elute proteins. Fifteen to 18 gel columns were pooled in an experiment. Eluates were dialyzed against distilled water for 2 days and mixed with the same volume of tissue culture medium 199 concentrated twice (Difco, Detroit, Michigan, U.S.A.) supplemented with the final concentration of 0.12 I.U./ml of bovine insulin and 1 µg/ml of cortisol. The final volume of each medium was made up to 5 ml. Mammary explants from nulliparous KA2* strain mice on day 8 of pregnancy were organ-cultured in this medium (Elias, 1959; Ito & Kohmoto, 1974). Lactogenic activity of each eluate was judged in histological sections as described by Kohmoto & Bern (1970).

Figure 1 shows a typical electrophoretic distribution in 7% acrylamide gel of radioactive leucine incorporated into proteins. Five radioactive peaks were observed. No radioactive peak was seen at the position of the albumin band. The medium from the first 2-day culture period had the highest radioactivity in every peak, and the third 2-day period medium showed the lowest radioactivity. An electrophoretic picture with five radioactive peaks indicated by arrows is presented at the bottom of Fig. 1. Results of organ culture of mammary glands in the medium containing eluates are summarized in Table 1. Eluates from two bands on the gels always had stimulatory effects on cultured mammary glands. These were eluates from gel segment no. 4-5 and no. 12-13 in Figure 1, which represent bands 1 and 4, whose mobilities were 0.85 and 0.33, respectively. Eluates containing proteins moving faster in gels than band 1 and moving more

* This strain was designated as KB in former papers (Yoshida, 1961; Ito & Kohmoto, 1974).
Table 1. Responses of KA2 mouse mammary glands to eluates from polyacrylamide gel columns.

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- No stimulation in mammary glands.
± Slight secretion, but not so much secretion as in control culture with prolactin (5 μg/ml).
+ Stimulated as much as control mammary gland culture with prolactin (5 μg/ml).

slowly than band 4 invariably gave negative reaction. Responses of mammary glands to eluates between bands 1 and 4 were variable.

The existence of placental lactogen is suggested for many mammalian species (cf. Yanai & Nagasawa, 1971; Foryth, 1973; Talamantes, 1975). Purification of the hormone has not yet been successful with three exceptions (Josimovich & MacLaren, 1962; Shome & Friesen, 1971; Fellows et al., 1974), probably because of the labile nature of this hormone (Grumbach & Kaplan; 1964). Linkie & Niswender (1973) observed luteotropic activity in crude extracts and in Sephadex eluates of 25,000–50,000 MW from rat placenta, but no luteotropic activity was associated with any visible electrophoretic bands. Talamantes (1973) reported that an amount of ultrafiltrate representing at least 25 mg of rat placental tissue was necessary to stimulate organ-cultured midpregnant mouse mammary gland significantly; a placental explant weighing approximately 1 mg was sufficient to stimulate mouse mammary glands in the organ coculture technique.

Radioactive peak observed in electrophoresis indicate that five proteins were synthesized and released from placental explants. It is not certain whether all of these are synthesized and normally secreted by the placenta or whether they are in part products of placental autolysis. However, since all three media showed the same five radioactive bands, it seems probable that these are consistently synthesized and released from placental explants. Suwa & Friesen (1969) related that two proteins were precipitated by an antiserum to human placental lactogen in the medium incubated with fragments of a human placenta, one of which was identified as human placental lactogen. In the present study, one of the five proteins synthesized and released from the mouse placental tissue whose electrophoretic mobility was slowest in 7% gel showed no lactogenic activity. This protein (band 5) is unlikely to be placental lactogen. The two proteins which invariably stimulated mammary tissues (band 1 and 4) probably represent one or two primary placental lactogen.

Tsushima et al. (1973) found the presence of placental growth hormone-like factor in the serum of pregnant monkeys and other mammals. From the data to date, one cannot conclude whether either of the lactogenic substances separated by electrophoresis is a placental growth hormone, inasmuch as the mammary glands of KA2 strain mice respond to ovine growth hormone as well as ovine prolactin (Ito & Kohmoto; 1974).
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References