In vitro Lactogen Production by Anterior Pituitaries from Various Species.* (30505)

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Lactogen has been detected in the pituitary gland of a number of species(1). Everett(2, 3) first demonstrated that removal of hypothalamic influence resulted in increased pituitary lactogen production in the rat. Other investigators(4,5) have reported the synthesis and release of large amounts of lactogen when rat pituitaries were cultured *in vitro*. *In vitro* lactogen production has been noted also by adenohypophyses from the mouse, guinea pig, rabbit, monkey(6), and man(7), but not from the pigeon(6). The investigation reported here was conducted to determine whether or not lactogen production occurred when pituitaries from rats, cattle, sheep, and pigeons were cultured *in vitro*.

Materials and methods. Culture techniques were similar to those previously reported(8). Anterior pituitaries (AP) were removed aseptically from 3-month-old, female, hooded Norway rats and divided into 6 pieces. Six to 10 AP were cultured per experiment at a temperature of either 35 ± 1.0°C or 37 ± 1.0°C for 3 days. Pituitaries from 3 calves and 1 sheep were so divided that each pituitary fragment was approximately 1 mm square, and 10 to 15 of these fragments placed in each culture dish. From 4 to 8 culture dishes were used per group. The anterior pituitary from calf no. 1 was divided into an outer and an inner region and cultured separately for 3 days at 35 ± 1°C. AP from 2 calves and 1 sheep (ram) were fragmentized and cultured separately for 3 days at 37 ± 1.0°C. AP from pigeons were divided into 6 pieces and a total of 3 AP were placed into each culture dish and cultured for 3 days at 37 ± 1°C.

Medium from each group was pooled and the lactogen content determined as previously described(9).† Lactogen content in precultured AP was determined in a manner similar to that used in determining the content in medium. Medium (20 ml) from pigeon cultures were dialyzed, lyophilized, and hydrated with 1.0 ml distilled water; this 20-fold concentrate was used for lactogen assays when cultured medium failed to give a lactogen response. Representative fragments of cultured AP tissue were examined histologically and degree of viability rated from zero to 4 as previously described(8). Differences between means were assessed statistically by means of Student's *t* test(10).

Results. Ten times more lactogen was synthesized by rat AP during a 3-day culture period than that introduced initially into the culture system. Pituitaries from cattle produced 2 to 5 times more lactogen than that present in precultured tissue, and viability ratings were lower than those noted for rat AP. Lactogen production from the outer region of a calf pituitary was higher than that from the inner region, but the difference was not significant. An anterior pituitary from a sheep was capable of considerable net synthesis of lactogen (6.6 ×) despite the fact that only a small amount of viable tissue was present at the end of a 3-day culture period. Pituitaries from pigeons were the only ones that did not synthesize and release lactogen *in vitro*. Lactogen could not be detected in the medium even after concentrating it 20 ×, and tissue survival was better than that noted for the other species (Table I).

Discussion. Previous reports(6,7) indicated that AP from a number of mammalian species were capable of lactogen production when removed from hypothalamic influence. Anterior pituitaries from cattle and sheep can be added to the list. The inability of pigeon AP to synthesize and release lactogen *in vitro* confirms a previous report(6). This latter observation may indicate the presence of a lactogen synthesizing and/or releasing factor(s) in the pigeon hypothalamus. Control of lac-

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† The prolactin used in this study was a gift from the Endocrinology Study Section, N.I.H.

TABLE I. Lactogen Production *in vitro* by Anterior Pituitaries of Various Species.

Specie	Avg AP wt/ culture dish (mg)*	Lactogen/100 ml medium (IU)*	Lactogen in medium/100 mg AP (IU)*	AP viability rating	Lactogen in 100 mg pre-cultured AP (IU)*	Net synthesis of lactogen *in vitro*†
Rat—						
Mature female	4.18‡	45.4‡	29.2‡	2.90‡	2.94 ± .28	9.9×
Cattle—female						
Calf No. 1						
Outer region	15.70 ± 3.10	51.5 ± 4.2	7.8 ± .6	1.75	—	2.5×§
Inner region	13.82 ± 3.14	53.2 ± 1.7	6.6 ± .2‖	1.75	—	2.1×§
Calf No. 2	10.27 ± .68	39.1 ± 6.3	10.3 ± 1.7	1.25	—	3.2×§
Calf No. 3	12.29 ± .80	71.6 ± 11.2	15.8 ± 3.0	1.50	3.18 ± .51	5.0×
Sheep—						
Mature ram	8.92 ± .59	64.5 ± 10.5	18.0 ± 3.0	1.00	2.83 ± .22	6.6×
Pigeon—						
Both sexes	8.30 ± .34	0¶	0¶	3.25	.44 ± .05	0

* Mean and standard error of mean.
† Calculated by dividing IU lactogen in 100 mg precultured AP into IU lactogen in medium/100 mg AP.
‡ Avg of 10 experiments for comparative purposes.
§ Estimation made using Cattle–female calf No. 3 precultured AP lactogen value.
‖ Not significantly different ($P > .10$) from outer region.
¶ Lactogen not detected in medium even after concentrating medium 20 times.

togen production in the pigeon pituitary has been thought to be unique(11) in that estrogen administration does not stimulate lactogen production as it does in mammals.

The lower viability estimates of cattle and sheep AP, compared with that of rat AP, may reflect specie differences in culture environment required for optimal tissue survival. It may be necessary, therefore, to determine optimal culture conditions for AP of each specie before undertaking a study of factors controlling lactogen production in that specie.

Summary. Anterior pituitaries from rats, cattle, sheep, and pigeons were cultured *in vitro* for 3 days. Synthesis and release of lactogen was 9.9 times greater for rat AP, 2.1 to 5.0 times greater for cattle AP, and 6.6 times greater for sheep AP than the amount of lactogen introduced initially into the culture system. Lactogen could not be detected in medium from pigeon AP cultures even after concentrating it 20 times.

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