parative studies indicated that isolates A-463 and F-205 were serologically identical. They were similar also as regards pathogenicity and cultural characteristics.

Summary. Pathogenic organisms were isolated from 2 outbreaks of a fatal septicemic disease of infant puppies and from dog kidney cells that degenerated spontaneously. The isolates were indistinguishable serologically and possessed characteristics of *Mycoplasma*. The pathogenic organisms were cytopathogenic for dog kidney cell cultures, and in inoculated puppies, produced pathological changes that resembled those seen in natural cases. Lesions consisted principally of necrosis and hemorrhage. The isolates were culturally and serologically distinct from recognized canine *Mycoplasma* species.

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Influence of Hypothalamic Fragments and Extracts on Lactogen Production *in vitro*.* (29711)

RICHARD R. GALA AND RALPH P. REECE

Department of Animal Sciences, New Jersey Agricultural Experiment Station, New Brunswick

A number of investigators, using different experimental techniques, have presented results which indicate that the hypothalamus plays a major role in control of anterior pituitary lactogen production. Hypophyseal stalk severance results in deciduomata formation by the traumatized uterus(1), maintenance of luteal function(2), and prevention of mammary gland involution(3). Anterior pituitary transplantation also results in deciduomata formation(4), initiation of mammary growth (5) and secretion(6), and retardation of mammary gland involution(7). Electrolytic lesions placed in the hypothalamus initiate lactation in estrogen primed rabbits(8), result in persistent diestrus and deciduomata formation(9), and retard mammary gland involution(9). Everett(4) suggested that the hypothalamus may produce a substance which inhibits lactogen production, and there are reports that addition of the hypothalamus to anterior pituitary cultures decreases lactogen production(10,11,12). The purpose of this study was to determine the effect of hypothalamic fragments and extracts from various sources on anterior pituitary lactogen production *in vitro*.

Materials and methods. Anterior pituitaries (AP) were obtained from 3-month-old, female, hooded Norway rats and divided into either 6 or 8 pieces. In one experiment AP were obtained from rats in mid-lactation. A piece from each anterior pituitary was placed in each group of an experiment until an equivalent of one AP was present per culture dish (6 to 10 dishes per group). The culture techniques were similar to those previously reported(13) and AP were cultured at either 35 ± 1.0°C or 37 ± 1.0°C for 3 days.

* Paper of the Journal Series, New Jersey Agric. Exp. Station, Rutgers, The State Univ. of New Jersey, Dept. of Animal Sciences, New Brunswick.

In Experiment 1, hypothalamic fragments (the equivalent of 2 hypothalamic regions) from estrous cyclic rats, 2 to 3 mm square, were cultured with an equivalent of 1 AP from an estrous cyclic rat in each dish. In Exp. 2, aqueous extracts of hypothalami from estrous cyclic rats were added to the medium to give an equivalent of 1.7 regions per dish in one group. In a second group an aqueous extract of cerebral cortex (equivalent to 1.7 hypothalamic regions) was added to the medium of each dish. In each group AP were from estrous cyclic animals. In Exp. 3, aqueous extracts of hypothalami from lactating rats were added to the culture medium to give an equivalent of 3 regions per culture dish, with AP from estrous cyclic animals. In Exp. 4, hypothalamic fragments from rats in mid-lactation were cultured with AP from the same animals. The equivalent of 2.0 hypothalamic regions were cultured per dish. Pituitaries from estrous cyclic animals and pituitaries from animals in mid-lactation, cultured alone, constituted two control groups. In Exp. 5, aqueous extracts of hypothalami from estrous cyclic rats were divided into 2 equal volumes, one of which was dialyzed for 6 hr at 3°C whereas the other was not. A sufficient volume of extract was added to the medium to give an equivalent of 3.0 hypothalamic regions per culture dish, with AP from estrous cyclic rats. Control groups, AP alone, were cultured simultaneously with experimental groups.

The lactogen content of the medium was determined as previously described(14). Representative pituitary fragments from each group were examined histologically and the degree of viable tissue rated from zero to 4 (13). Differences between means were assessed statistically using Student's *t* test(15).

Results. Culturing of anterior pituitaries with hypothalamic fragments from estrous cyclic rats resulted in a 27.6% decrease in lactogen production per 100 mg AP tissue (Exp. 1). In Exp. 2, lactogen production by pituitaries cultured with an aqueous extract of hypothalami from estrous cyclic rats was 34.3% lower than that of controls. The addition of an aqueous extract of cerebral cortex to the culture medium resulted in a 14.6% decrease in lactogen production. In the third experiment the addition of an aqueous extract of hypothalami from lactating rats to AP from estrous cyclic rats resulted in a 33.5% decrease in lactogen production. In Exp. 4, lactogen production by pituitaries from lactating rats cultured with hypothalamic fragments from lactating rats was 17.2% less than that of AP from lactating rats and 27.3% less than that of AP from estrous cyclic rats. Among experiments there was considerable variation in lactogen production by control cultures, thus indicating the importance of culturing simultaneously control and experimental tissue. In the fifth experiment, the addition of dialyzed and non-dialyzed aqueous hypothalamic extracts from estrous cyclic rats did not influence lactogen production, a result that cannot now be explained.

Neither the manner in which the hypothalamus was introduced into the culture system (fragment or aqueous extract) nor the source of the hypothalamus (estrous cyclic or lactating rats) altered the hypothalamic effect on lactogen production. Data, therefore, were assembled into control and hypothalamic groups and mean differences tested for significance. Lactogen production per 100 mg AP tissue by pituitaries cultured with hypothalamus was 22.3% less than that of AP cultured alone, and the difference was highly significant. Postcultured AP weights, lactogen content of cultured AP tissue, and AP viability ratings of the experimental groups were not significantly different from those of the control groups (Table I).

Discussion. The significant decrease in anterior pituitary lactogen production *in vitro* upon the addition of either hypothalamic fragments or hypothalamic extracts has been noted by other workers(10,11,12). Pasteels (11) demonstrated further, that the removal of hypothalamic fragments and addition of fresh medium resulted in a significant increase in lactogen production over the previous period when hypothalamic fragments were present. Since the observation that cerebral cortical extracts did not decrease lactogen production to the extent noted for hypothalamic extracts has been reported also by

TABLE I. Influence of Hypothalamic Fragments and Aqueous Extracts on Anterior Pituitaries Cultured *in vitro*.

Exp No.	Tissue cultured*	Avg AP wt postculture (mg) †‡	Lactogen/100 ml medium (IU) †	Lactogen in medium/100 mg AP (IU) †	Lactogen in 100 mg cultured AP (IU) †	AP viability rating
1	Control—AP, ECR	4.03 ± .21	48.1 ± 7.9	32.3 ± 5.7	1.40 ± .36	2.75
	2 HF + AP, ECR	3.74 ± .25	32.9 ± 4.4	23.4 ± 3.1	1.31 ± .20	3.00
2	Control—AP, ECR	4.13 ± .14	34.6 ± 7.8	23.3 ± 5.2	1.44 ± .30	2.50
	Ext. CC + AP, ECR	4.63 ± .45	32.4 ± 1.9	19.9 ± 1.2	1.75 ± .38	2.75
	Ext. 1.7H + AP, ECR	4.08 ± .11	22.5 ± 2.3	15.3 ± 1.6	1.88 ± .12	2.25
3	Control—AP, ECR	4.45 ± .22	46.9 ± 4.4	29.9 ± 2.8	2.88 ± .33	3.25
	Ext. 3H, LR + AP, ECR	4.39 ± .14	31.5 ± 3.9	19.9 ± 2.4	1.93 ± .56	2.50
4	Control—AP, ECR	3.70 ± .21	33.6 ± 5.0	23.8 ± 3.5	1.35 §	3.00
	Control—AP, LR	4.63 ± .35	36.1 ± 3.2	20.9 ± 1.9	2.25 ± .86	3.00
	2 HF, LR + AP, LR	4.48 ± .29	29.0 ± 5.4	17.3 ± 3.2	1.33 ± .27	2.50
5	Control—AP, ECR	4.35 ± .21	31.2 ± 3.5	19.6 ± 2.2	1.49 ± .27	3.00
	Ext. 3H, ECR, ND + AP, ECR	3.96 ± .20	29.8 ± 3.2	20.4 ± 2.2	2.21 ± .61	3.25
	Ext. 3H, ECR, D + AP, ECR	4.51 ± .18	30.5 ± 3.6	18.4 ± 2.2	1.14 ± .18	2.50
Avg	Control, AP	4.32	38.9	25.8	1.71	2.90
Avg	H Ext. or HF + AP	4.13‖	29.1	19.3¶	1.73‖	2.70‖

* AP = anterior pituitaries; ECR = estrous cyclic rats; HF = hypothalamic fragments; Ext. CC = aqueous extract cerebral cortex; Ext. H = aqueous extract hypothalamus; LR = lactating rat; ND = non-dialyzed; D = dialyzed.

† Mean and S.E. of mean.

‡ Six to 10 AP cultured per group.

§ Two out of 2 pigeons responded the same.

‖ Not significantly different ($P>.10$) from control.

¶ Significantly less ($P<.01$) than control.

other investigators(11,12), Everett's(4) suggestion that the hypothalamus produces a lactogen-inhibitory factor appears to be tenable.

The lactogen-inhibitory effects of hypothalamic fragments and extracts have been demonstrated to date only for hypothalamus from estrous cyclic animals(10,11,12). Data reported here indicate that either hypothalamic aqueous extracts or hypothalamic fragments from lactating rats also possess the lactogen-inhibitory factor.

Although the lactogen content in the medium of hypothalamic groups was lower than that of controls, the decrease was not due to lack of lactogen release, since the lactogen content of AP tissue cultured with hypothalamus was not significantly different from that of controls. Meites *et al*(12) reported that, in short-term cultures (2 hr), addition of hypothalamus significantly reduced lactogen content not only in the medium, but also of the cultured glands. This lack of agreement may be explained by the difference in the duration of culture.

Summary. Anterior pituitaries from estrous cyclic and lactating, hooded Norway rats were cultured in synthetic medium (Mixture 199) with hypothalamic extracts or fragments for a period of 3 days at a 300 cc/min gas flow of 95% O_2-5% CO_2. In 5 experiments, lactogen production was decreased an average 22.3% by the addition of hypothalamus. Hypothalamic extracts and hypothalamic fragments from either estrous cyclic or lactating animals were capable of inhibiting pituitary lactogen production *in vitro*. Addition of cerebral cortical extracts to the culture system did not significantly alter lactogen production. Lactogen content of cultured pituitary tissue and AP viability ratings of hypothalamic groups did not differ significantly from those of controls.

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Cytological Detection of Parathyroid Hormone by Immunofluorescence. (29712)

GARY K. HARGIS, VINCENT J. YAKULIS, GERALD A. WILLIAMS, ARTHUR A. WHITE
(Introduced by Paul Heller)

Research Laboratory and Medical Service, Veterans Administration West Side Hospital, and Department of Medicine, University of Illinois College of Medicine, Chicago

This laboratory(1) previously reported the production of a specific antiserum to bovine parathyroid hormone (PTH). This antiserum was used for immunochemical detection of PTH in crude and purified extracts of bovine parathyroid glands (PTG). The limited quantities of human and rat parathyroid tissue available for extraction prevented satisfactory comparative immunochemical study of the PTH of these species.

The fluorescent antibody technic(2) allows the immunochemical study of cellular substances in tissue section and therefore requires only a small quantity of tissue. This technic has been used by others(3-8) to detect and localize several of the polypeptide hormones in cells of endocrine glands.

The present study, utilizing the fluorescent antibody technic, was undertaken to determine 1) the feasibility of this technic for detecting PTH in tissue, 2) the species-specificity of PTH, 3) the cellular site of PTH synthesis and/or storage in the PTG.

Materials and methods. Rabbit anti-bovine parathyroid hormone serum(1) was conjugated with fluorescein isothiocyanate by the method of Rinderknecht(9). The gamma globulin fraction, containing the fluorescent antibody, was isolated with DEAE-cellulose by the method of Baumstark(10). This fluorescent anti-PTH globulin was absorbed with bovine serum and thyroid tissue as previously described(1).

Specimens of bovine and rat parathyroid, thyroid and lymph node were obtained immediately after death. Similar tissue specimens were obtained from 2 human subjects (55- and 60-year-old males with no evidence of abnormal parathyroid function) at autopsy 4 hours after death. Each specimen was quick-frozen in absolute alcohol precooled in an acetone-dry ice bath. The specimens were subsequently embedded in paraffin, sectioned at 3-5 μ thickness, prepared, stained and mounted by the technic of Saint-Marie(11), with slight modifications. The tissue sections were reacted with fluorescent anti-PTH globulin in a moist chamber on a rotating table at room temperature for 4 hours. In control studies, the sections were allowed to react with a) non-conjugated anti-PTH serum prior to application of the fluorescent anti-PTH globulin, or b) fluorescent anti-PTH globulin which had been previously absorbed with bovine PTH. In initial studies the fluorescent anti-PTH globulin was absorbed with activated charcoal(12) or liver homogenate(13). However, this absorption procedure did not alter the staining characteristics with any of the tissues, and was therefore discontinued.

The tissue sections stained with fluorescent