ponding to both soluble and insoluble collagen fractions was demonstrated biochemically in the connective tissue of many organs, a reaction occurring with antibodies against the collagen fractions as well as with those against the acetic acid extract was to be expected. Consequently, only those fractions such as the neutral salt-soluble and the insoluble fractions, which exhibit no cross immunological reactions appear suitable for the histo-immunological detection of collagen. It is suggested that the technique used in the present study may provide another approach for the microscopical localization of the precursors of collagen structures during development of connective tissue.

Summary. The soluble and insoluble fractions of collagen from chicken leg tendon were used as antigens to induce corresponding antibodies in adult rabbits and guinea pigs. Antibodies were checked immuno-serologically and the globulins or its gamma fraction labeled with fluorescein isothiocyanate and purified with Sephadex. Adequately controlled Coons' technique was used in order to detect the presence of the antigen in different types of connective tissue of adult chickens. It was observed: 1) as previously demonstrated, there was cross reaction between the neutral-soluble and the citrate-soluble fractions, whereas a direct reaction occurred with the insoluble collagen fraction, 2) the fluorescent antibodies against these different fractions reacted similarly with the collagen fibers and bundles but not with reticulin and elastic fibers, mucopolysaccharides, basement membranes, nor with matrix of cartilage and bone, 3) intensity of fluorescence was higher with the fibers of dense connective tissue as compared with loose or mucoid type of connective tissue.

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The Presence of Prolactin Inhibiting Factor (PIF) in Extracts of Beef, Sheep and Pig Hypothalami.* (29839)

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Talwalker, Ratner and Meites(1) clearly demonstrated the presence of a factor in rat hypothalamic extracts which inhibited the synthesis and release of prolactin *in vitro*. This was provisionally designated as prolactin inhibiting factor (PIF). Schally, Meites, Bowers and Ratner(2) showed that preparations of hypothalamic luteinizing hormone re-

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TABLE I. Release of Prolactin *in vitro* from Pituitaries of Female and Male Rats in KRBG and Medium TC 199.

Rats	Medium	Reece-Turner response*		Prolactin I.U./100 mg pit	
		Left halves	Right halves	Left halves	Right halves
♂	KRBG	1.62 ± .17	1.71 ± .20	1.48	1.27
"	TC 199	1.72 ± .11	1.71 ± .08	1.41	1.27
♀	KRBG	1.79 ± .13	1.88 ± .10	1.70	2.08
"	TC 199	1.97 ± .12	1.98 ± .19	2.30	2.73

* Mean ± S.E.

leasing factor (LRF) of bovine or ovine origin did not have an inhibitory effect on the prolactin release *in vitro, i.e.,* that LRF and PIF are not the same. The present investigation was undertaken to determine whether extracts from hypothalami of domestic animals have PIF activity.

Materials and methods. Fragments of beef, sheep and pig hypothalamic tissue containing mainly the pituitary stalk and the median eminence (SME) area were collected on dry ice and lyophilized. The fragments were then defatted with acetone and petroleum ether and extracted with 2 N acetic acid at 8°C. The extracts were quickly heated to boiling, chilled on ice and lyophilized. The details of extraction will be reported elsewhere(3). Lyophilized hypothalamic extracts stored in high vacuo over pellets of NaOH were extracted with incubation medium. The suspension was spun at 15,000 rpm at 4°C and the supernatant was added to incubated pituitaries.

Incubations. Pituitary incubations were carried out by the method of Saffran and Schally(4) and in some cases as modified by Talwalker, Ratner and Meites(1).

Female rats of Holtzman Sprague-Dawley strain, 280-350 g, were primarily used as pituitary donors in the experiments; however, in some experiments male rats of the same strain were utilized. Each anterior pituitary was cut in half(4) and transferred to a 30 ml Pyrex beaker containing 2.5 ml Krebs-Ringer bicarbonate medium with 200 mg% glucose (KRBG) or in some cases 2.5 ml volume of synthetic protein free medium TC 199 (Difco Laboratories, Detroit, Mich.). A total of 9 pituitary halves were placed in each flask, one flask serving as control and an equal number of anterior pituitary halves from the same rats being incubated with hypothalamic extracts. The incubation time was 2 hours.

Prolactin assay. Prolactin activity of the samples was determined essentially by an intradermal pigeon crop assay method(5) by a paired assay procedure(1,2). Six white Carneau squabs (Palmetto Pigeon Plant, Sumter, S. C.) were used to assay each sample. An NIH preparation of ovine prolactin P-S 5, 17 IU/mg was used as standard.

Results and discussion. Results of incubation of pituitaries from male or female rats in KRBG and medium TC 199 are summarized in Table I. On incubation pituitary halves released similar amounts of prolactin when tested against their corresponding halves. Male and female pituitaries released comparable amounts of prolactin. The release of prolactin *in vitro* in medium TC 199 was comparable to that in KRBG medium in short term incubations.

Subsequent incubations were carried out in KRBG since the release of prolactin was similar in KRBG or medium TC 199. At least 2 experiments were performed on each extract and results were in agreement.

Addition of ovine hypothalamic extracts to the incubation media significantly reduced levels of prolactin in the medium (Table II). The same Table shows that in 4 experiments addition of bovine hypothalamic extracts caused a 4-5-fold reduction of the levels of prolactin in the medium. Due to the variability of the prolactin assay only 2 experiments are statistically significant. Similarly in 4 experiments pig hypothalamic extracts caused a 4-6-fold inhibition of prolactin release, but again, due to a high standard error of the assay only 2 experiments are statistically significant. The results of these experiments suggest that hypothalamic extracts of ovine, bovine and porcine origin have an

TABLE II. Effect of Hypothalamic Extracts of Porcine, Bovine and Ovine Origin on the Release of Prolactin *in vitro*.

Treatment	Dose SME fragments	Reece-Turner response units* Control	Experimental	Prolactin I.U./100 mg pit Control	Experimental	P†
sheep SME	1	2.2 ± .11	1.5 ± .10	3.00	.49	.001
" "	1	1.6 ± .11	1.0 ± .23	.76	.11	.05
beef SME	.3	1.9 ± .10	1.5 ± .19	1.40	.37	.05
" "	.3	2.0 ± .21	1.3 ± .15	1.99	.41	.05
" "	.5	2.0 ± .2	1.4 ± .15	2.68	.5	.05
" "	.5	2.0 ± .16	1.5 ± .23	2.29	.53	.1–.2
pig SME	1	2.2 ± .31	1.6 ± .29	3.05	.60	.1–.2
" "	1	2.2 ± .16	1.7 ± .12	3.82	.90	.05
" "	.5	1.9 ± .17	1.4 ± .15	1.69	.43	.05
" "	.5	2.0 ± .18	1.4 ± .2	2.25	.42	.05–.1

* Mean ± S.E. † P experimental *vs* control in Reece-Turner Units.

inhibitory effect on prolactin release *in vitro*.

Nicoll and Meites(6) showed that neither oxytocin nor vasopressin influenced prolactin production by rat anterior pituitary *in vitro* indicating that neurohypophyseal hormones are not involved in regulation of prolactin secretion. Talwalker(1) showed that decreases in prolactin activity they observed are not due to inactivation of prolactin and that PIF activity is absent from brain cortex extracts.

These results corroborate and extend the findings of Talwalker, Ratner and Meites(1). The presence of PIF in extracts of hypothalami of large domestic animals makes this activity amenable to purification.

Summary. Rat anterior pituitary halves incubated *in vitro* release prolactin at a comparable rate in 2 incubation media. Addition of hypothalamic extracts of bovine, ovine or porcine origin inhibited release of prolactin *in vitro*.

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Analgesic and Psychotomimetic Properties of Dexoxadrol.* (29840)

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Dexoxadrol (d-2,2-diphenyl-4-(2-piperidyl)-1,3-dioxolane hydrochloride) has been studied extensively in the laboratory, where it displays a combination of depressant and stimulant effects on the central nervous system (unpublished data, Upjohn Co.). These include abolition of the righting reflex, anticonvulsant effects, blocking or depression of the EEG alerting reaction, and parasympathetic blockade. Of particular interest are its high degree of local anesthetic activity and the paradoxical fact that the drug is capable of inducing in mice a condition of "hyperacuity," as demonstrated by an increased response to thermal stimuli and increased writhing after intraperitoneal phenylquinone(1).

Our interest was aroused by reports of clinical pharmacologic experiments wherein

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