TABLE I. Distribution of Electrolytes and Water in Aorta Wall.*

	No. of rats	H_2O (g)		Na (meq)		K (meq)	
		Extra-cell	Intra-cell	Extra-cell	Intra-cell	Intra-cell	
Normal	18	18.0 ±.4†	174 ± 1.0	23.4 ±.3	1.4 ±.08	11.0 ±.13	
Hypertensive + stock diet	16	22.4 ±.6	217 ± 1.4	28.2 ±.7	2.9 ±.10	14.9 ±.27	
Hypertensive + diazoxide	14	20.9 ±.7	210 ± 1.8	27.1 ±.5	3.1 ±.11	12.3 ±.21	P <.01
Hypertensive + quinethazone	16	21.3 ±.9	206 ± 2.0	26.8 ±.8	2.8 ±.18	12.0 ±.32	P <.01

* Per 100 g fat-free dry wt. † Standard deviation.

Numerous reports from this laboratory have indicated that under a number of conditions there has been a positive correlation between blood pressure level and concentration of potassium in arterial tissue(3). It has been suggested that the blood pressure level of renal hypertensive animals is regulated by this cation acting on arterial tone and thus influencing peripheral resistance. This hypothesis appears to be supported by the evidence that diazoxide(2) as well as other thiazides(4) induce a lowering of peripheral vascular resistance in association with a reduction in aorta wall potassium content(1). It is, therefore, deduced that the anti-hypertensive action of thiazides may be mediated through this effect on arterial muscle potassium. Likewise, quinethazone which differs chemically and pharmacologically from the thiazides may exert its anti-hypertensive effect through a similar mechanism.

Summary. Quinethazone lowers blood pressure of renal hypertensive rats in association with a decrease in potassium but no change in water or sodium contents of aorta wall. The anti-hypertensive effect of quinethazone, like the thiazides, may be mediated through this electrolyte alteration in arterial muscle.

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Influence of Type and Level of Gas Environment on Anterior Pituitary Lactogen Production *in vitro*.* (28695)

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Anterior pituitaries (AP) freed from hypothalamic control and cultured *in vitro* are capable of considerable net synthesis of lactogen(1,2,3). Although Meites and co-workers (1,4) reported greater lactogen production by pituitaries cultured in an environment of 95% O_2 – 5% CO_2 than by pituitaries cultured in an air environment, they did not indicate the level of gas flow. The purpose of this investigation was to determine the influence of type of gas environment on lactogen production and level of 95% O_2 – 5% CO_2 gas flow that resulted in maximal lactogen production *in vitro*.

Materials and methods. Primary anterior pituitary explants from 3-month-old, female, hooded Norway rats were cultured *in vitro*

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as described by Meites and co-workers(1). Pituitaries were divided into 6 pieces and one pituitary was placed in each culture dish. A total of 5 or 6 dishes was cultured per group for a period of 3 days at 35± 1.0°C. The culture medium was chemically defined Mixture 199; to it was added 0.1 mg of zinc-free insulin†, 0.2 mg of streptomycin, and 0.068 mg penicillin/ml of medium. A 2.8% $NaHCO_3$ solution was added to the medium at the level of 2.5 ml/25 ml and 3.0 ml of medium were added per culture dish.

In the initial study anterior pituitaries were cultured in an environment consisting of either air, pure O_2 at a flow of 300 and 900 cc/min, or 95% O_2 – 5% CO_2 at a flow of 300 cc/min. In a subsequent study, the 95% O_2 – 5% CO_2 gas environment was metered at the rate of either 30, 150, 300, or 900 cc/min. In the latter study humidification of the culture system was accomplished by bubbling the gas mixture through distilled water prior to its entrance into the culture chamber, whereas in the initial study no humidification was attempted.

Medium in each group was pooled and the lactogen content determined by the assay method of Reece and Turner(5). Pooled medium was injected intradermally over the right crop gland of mature pigeons at a level of 0.05 ml/day for 4 days, and 1.25 μg lactogen‡ (0.01875 IU) in the same volume was injected over the left crop gland. On the fifth day the pigeons were sacrificed and the degree of proliferation rated visually. A total of 5 to 9 pigeons was used per group. Lactogen content of cultured pituitary tissue was estimated in a manner similar to that in medium, and an aqueous suspension of 1.25 mg/0.05 ml was injected daily over the right crop gland. A total of 3 to 5 pigeons was used per group.

Representative fragments of cultured anterior pituitary tissue from each group were fixed in 10% neutral formalin and stained with Lillie's azure-eosin. Viability estimates were made and ratings were assigned from 0 to 4.0. A tissue receiving a rating of 4.0 was comparable in cellular viability and intensity of stain to that observed for precultured anterior pituitary tissue. A tissue receiving a rating of zero was completely necrotic and had little affinity for the stain. Data were analyzed by Student's *t* test.

Results. The culturing of rat anterior pituitaries (AP) *in vitro* in a 95% O_2 – 5% CO_2 gas environment at a flow of 300 cc/min resulted in nearly twice the lactogen production of that in air (Table I). Lactogen production in a pure O_2 gas environment at a flow of 300 and 900 cc/min was 1/3 and 1/6 respectively, of that noted for 95% O_2 – 5% CO_2. Lactogen content in cultured AP tissue from the 95% O_2 – 5% CO_2 and air groups followed a trend similar to that of lactogen production in medium, and lactogen could not be detected in pituitary tissue cultured in pure O_2.

Histological comparisons of AP cultured in the various gas environments with precultured pituitaries indicated that a 95% O_2 – 5% CO_2 gas environment at 300 cc/min flow was capable of maintaining tissue in a highly viable state (Table I; Fig. 1 and 2). Histological examination of anterior pituitaries cultured in pure O_2 revealed almost complete necrosis, while culturing in air maintained tissue integrity better than pure O_2 but not as well as 95% O_2 – 5% CO_2 (Table I; Fig. 1, 2, 3, and 4).

Lactogen production by AP cultured in 95% O_2 – 5% CO_2 at a gas flow of 30 cc/min was no greater than that in air; cultured pituitary lactogen concentration, however, was higher than that in air. In 4 experiments the lactogen production by AP cultured in either 150 or 300 cc/min flow of 95% O_2 – 5% CO_2 were not significantly different from each other. Anterior pituitary viability rating and lactogen content in cultured tissue were also similar for the two groups. Pituitaries cultured at the highest flow of 95% O_2 – 5% CO_2, 900 cc/min synthesized and released 35% less lactogen than AP cultured at the 150 or 300 cc/min flow. Lactogen content in cultured tissue was also lower, but the viability rating was similar (Table II).

† Zinc-free insulin (20 units/mg) kindly supplied by Eli Lilly and Co., Indianapolis, Ind., through the courtesy of Dr. Otto K. Behrens.

‡ Ovine prolactin (15 IU/mg) received as a gift from the Endocrinology Study Section, N.I.H.

Humidification of the gas environment in the second study (level of 95% O_2 – 5% CO_2) decreased medium loss from 25-35%, noted in the initial study, to less then 10%, and indicated that these losses were due to evaporation. Prevention of medium loss provided a more constant and uniform environment for the maintenance of anterior pituitary viability *in vitro*.

Discussion. Previous studies(1,3,4) indicated that a considerable net synthesis of lactogen occurred when anterior pituitaries were cultured *in vitro*. We have found that the precultured AP lactogen content was 2.94 IU/100 mg tissue. Considering the amount of lactogen detected in the medium per 100 mg AP tissue for AP cultured in a 95% O_2 – 5% CO_2 gas environment, 6.5 to 7.8 times more lactogen was synthesized and released than initially introduced into the system. This represents a considerable net synthesis of lactogen and substantiates the reports of Meites and co-workers that once freed from hypothalamic influence, the anterior pituitary is capable of considerable lactogen synthesis.

The lactogen concentration in cultured AP

TABLE I. Influence of the Type of Gas Environment on Anterior Pituitaries Cultured *in vitro*.

Parameters	Air	Pure O_2		95% O_2–5% CO_2
		300 cc/min	900 cc/min	300 cc/min
Anterior pituitaries				
No./group	5	5	5	5
Avg wt postculture (mg)*	5.30 ± .20	6.35 ± .72	8.00 ± .72‡	5.50 ± .83
Lactogen in 100 mg cultured AP (IU)*	.67 ± .04	0	0	1.25 ± .39
No. pigeons/AP tissue assay†	2/2	0/3	0/5	3/3
AP viability rating	2.0	0	1.0	3.5
Medium				
Loss in volume (%)	22.7	26.7	37.3	30.6
Lactogen/100 ml media (IU)*	25.2 ± 3.3	18.2 ± 3.2	14.1	50.3 ± 6.2
Lactogen in media/100 mg AP (IU)*	11.0 ± 1.4§	6.3 ± 1.2‖	3.3	19.0 ± 2.3
No. pigeons/media assay†	5/5	5/7	1/5	5/5

* Mean and standard error of mean.
† No. pigeons responded/No. pigeons used.
‡ Significantly higher ($P < .05$) than other groups.
§ Significantly lower ($P < .025$) than 95% O_2 – 5% CO_2 group.
‖ Significantly lower ($P < .01$) than 95% O_2 – 5% CO_2 group.

TABLE II. Influence of the Level of 95% O_2 – 5% CO_2 Gas Flow on Anterior Pituitaries Cultured *in vitro*.

Level of 95% O_2 – 5% CO_2 gas flow	Exp No.	Avg AP wt postculture*†	Lactogen 100 ml medium (IU)*	Lactogen in medium/100 mg AP (IU)*	Lactogen in 100 mg cultured AP (IU)*	AP viability rating
30 cc/min	1	7.20 ±1.20	25.0 ± 3.6	7.8 ± 1.1	1.77 ± .38	1.00
150 cc/min	1	4.34 ± .35	48.4 ± 2.5	30.4 ± 3.9	2.33 ± .54	3.00
	2	4.22 ± .12	28.9 ± 3.3	19.2 ± 2.2	1.88 ±1.10	3.50
	3	3.92 ± .23	31.9 ± 4.2	22.5 ± 2.9	—	3.00
	4	3.35 ± .26	23.6 ± 3.6	19.2 ± 2.9	—	3.00
	Avg	3.96§	33.2	22.8§	2.11§	3.13§
300 cc/min	1	3.60 ± .27	29.5 ± 7.9	21.1 ± 5.6	1.02 ± .26	2.50
	2	3.98 ± .10	36.0 ± 7.3	24.6 ± 5.0	2.50 ± .36	2.50
	3	4.15 ± .30	35.0 ± 4.3	22.6 ± 1.6	2.25 ±0 ‡	2.50
	4	4.10 ± .21	25.1 ± 4.1	17.6 ± 2.8	—	3.25
	Avg	3.96	31.4	21.5	1.92	2.69
900 cc/min	1	4.18 ± .25	32.5 ± 3.1	14.6 ± 1.4	1.12 ± .39	3.00

* Mean and standard error of mean.
† Five or 6 AP cultured per experiment.
‡ Two out of 2 pigeons gave the same response.
§ Not significantly different ($P > .10$) from 300 cc/min group.

tissue was found to be highest for pituitaries cultured in an environment of 95% O_2 – 5% CO_2. The lactogen concentration, however, was still lower (28 to 58%) than that of

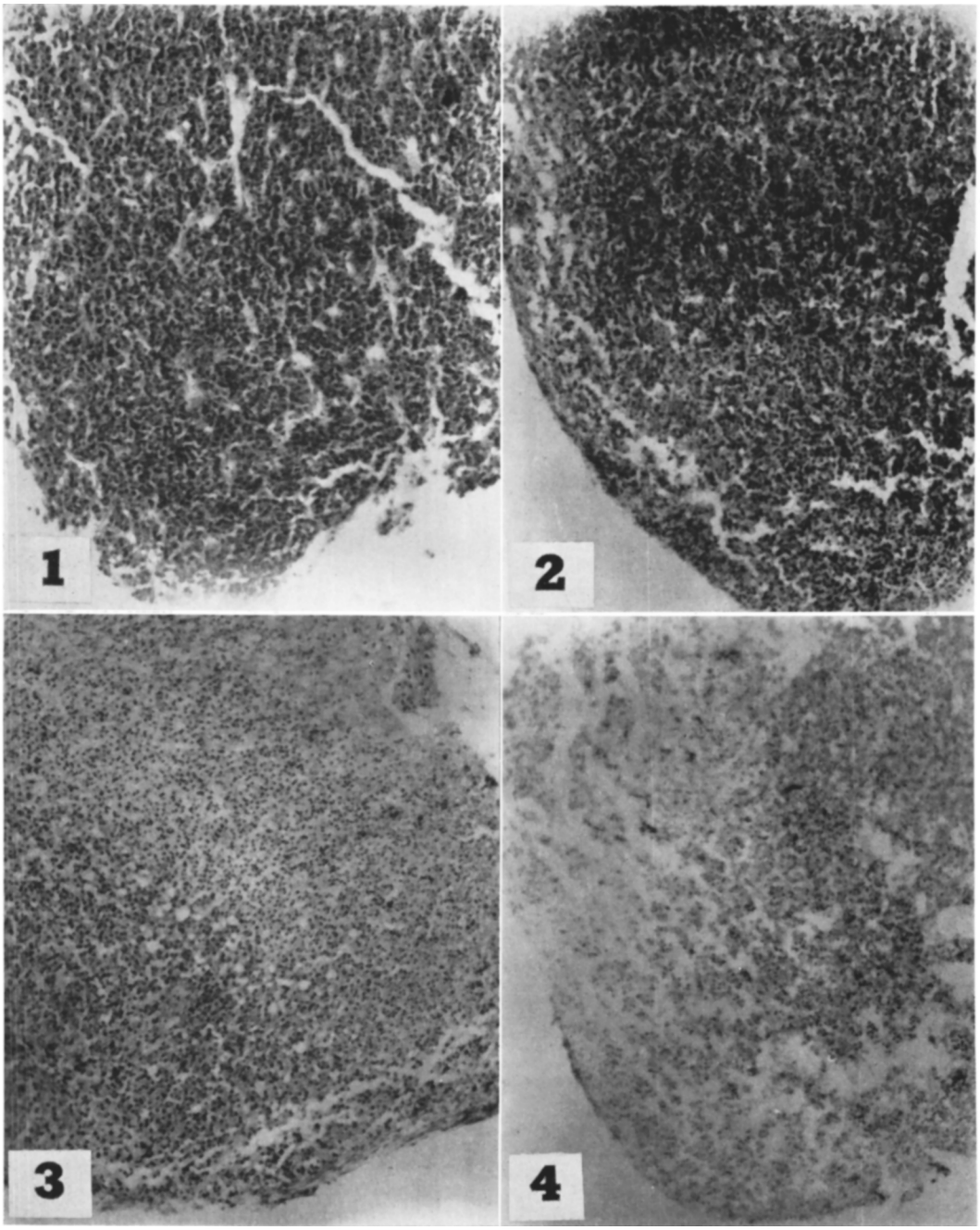

FIG. 1-4. Photomicrographs of histological sections from rat anterior pituitaries (AP); 10% neutral formalin; Lillie's azure-eosin; 8 μ: (1) Precultured AP. (2) Anterior pituitary cultured for 3 days in a gas environment of 95% O_2-5% CO_2 at a flow of 300 cc/min. AP tissue maintained in a highly viable state. (3) Anterior pituitary cultured for 3 days in an air environment. Note center region of necrosis and outer region of viable tissue. (4) Anterior pituitary cultured for 3 days in a gas environment of pure O_2 at a flow of 900 cc/min. Areas of extensive necrosis are evident. × 120.

precultured pituitaries. This decrease may be due to either tissue necrosis which occurred during culture, or to removal of the hypothalamic influence. Histological examination, however, indicated that cultured pituitaries were maintained in a highly viable state. It has also been noted that anterior pituitaries homotransplanted to the kidney capsule had ½ to ⅓ the lactogen concentration of non-transplanted pituitaries (Gala and Reece, unpublished). It is believed that the decrease in lactogen concentration was due to removal of the hypothalamic influence.

A culture environment of pure O_2 appeared to be toxic to anterior pituitary tissue. This toxicity was reflected not only in cellular necrosis, but also in lactogen production and in lactogen concentration of the cultured tissue. Addition of only 5% CO_2 to the gas environment was capable of overcoming the toxicity of pure O_2 noted; however, the level of flow of 95% O_2 – 5% CO_2 mixture must also be considered. Levels below 150 cc/min were no better than air, and above 300 cc/min there was a decrease in lactogen production. Lactogen production and cellular viability for AP cultured as primary explants are therefore dependent upon the type of gas environment and level of gas flow.

Summary. Anterior pituitary explants from rats, cultured *in vitro* in the chemically defined Mixture 199 for 3 days, were capable of synthesizing and releasing into the medium 6 to 8 times more lactogen than initially introduced into the culture system. Lactogen production and cellular viability were dependent upon the gas environment and a 95% O_2 – 5% CO_2 gas mixture at a flow of either 150 or 300 cc/min was optimum. A pure O_2 environment did not support lactogen production and appeared to be toxic to anterior pituitary tissue. Culturing AP in air resulted in half the lactogen production noted for AP in 95% O_2 – 5% CO_2 and tissue survival was poor.

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Stimulation of Ovulation by Purified LH-Releasing Factor (LRF) in Animals Rendered Anovulatory by Hypothalamic Lesion.* (28696)

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There is now considerable evidence that extracts of the median eminence region of the hypothalamus contain a substance which stimulates the release of ovulation hormone (LH)(1,2,3,4). We have recently reported partial purification of this substance (LRF, for LH-releasing factor) using the ovarian ascorbic acid depletion method as a test of endogenous release of LH(5). To validate any possible physiological significance of these observations we had to demonstrate that this material (LRF) would stimulate secretion of LH in animals in which the cyclic release of LH was blocked by a suitable hypothalamic lesion, the most specific criteria for LH-activity being used, *i.e.*, ovulation with direct observation of tubal ova and subsequent formation of corpora lutea.

Materials and methods. Experiment I. Forty-two female rats, Wistar origin (Duterme Farms, Condé s/Huisne, France) B. W. 170-200 g were utilized here. In each of them a hypothalamic lesion was produced by a high frequency coagulation between the

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