of kanamycin has no influence on the fertility rate of rats.

2) Do auditory stimuli influence fertility in deafened rats? Fifty-eight female rats treated with kanamycin were exposed to auditory stimuli during a 4 days copulation period and killed on the 20th day of the experiment. Forty-three rats were found to be pregnant (74.1%), producing 260 offspring, an average of 6 young per rat. The experiments show that auditory stimuli during the copulation period do not cause a decrease in fertility of deafened rats in contrast to normal animals.

3) Do auditory stimuli block pregnancy in deafened rats? Forty deaf female rats were housed together with males, each couple in a separate cage, for a 4 day copulation period. On the 7th to 9th day the pregnant females were exposed to auditory stimuli and killed on the 20th day after the start of experiment. Twenty-eight deaf females were found to be pregnant (70% fertility), producing 169 litter (5.7 litter per rat). These findings demonstrate that auditory stimuli during pregnancy do not disturb the course of pregnancy in deafened rats, whereas in normal rats, as mentioned above, blockage of pregnancy occurs.

Summary. Auditory stimuli in the audiorange are ineffective in inducing infertility and blockage of pregnancy in deaf rats by damage of the organ of Corti in the inner ear following administration of kanamycin.

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In vitro Lactogen Production by Anterior Pituitaries from Lactating Rats and Estrous Cyclic Rats.* (29327)

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Reece and Turner(1) reported a 2-fold increase in anterior pituitary lactogen content in rats following parturition, and a similar increase has been noted for mice(2), guinea pigs(1,3,4) and rabbits(4,5). Meites *et al* (6) found that anterior pituitaries from lactating rats cultured *in vitro* produced, on a per gland basis, twice as much lactogen as did glands from virgin females 3-4 months old. The purpose of the present study was to determine lactogen production by pituitaries from lactating rats and estrous cyclic rats during first 3 days, the second 3 days, and the third 3 days of *in vitro* culture.

Materials and methods. Anterior pituitaries (AP) were obtained from 3-month-old, hooded Norway rats lactating an average of either 4 or 14 days (10 per group) and cultured simultaneously with AP from estrous cyclic rats (10 per group). Estrous cyclic rats were sacrificed without reference to stage of estrous cycle and were the same age as the lactating rats, but 20 to 30 g lighter in body weight.

Culture techniques were similar to those reported by others(6). Anterior pituitaries were divided into 4 pieces, one gland per dish, and cultured at 35 ± 1.0°C. Gas environment was humidified 95% O_2-5% CO_2 at a

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flow of 300 cc/min, and duration of culture was 9 days. To the culture medium, the chemically defined Mixture 199, were added 0.2 mg streptomycin and 0.068 mg penicillin/ml and 2.0 ml of 2.8% $NaHCO_3$/25 ml. Three ml of medium were added per culture dish and the medium was changed after 3 and 6 days of culture.

Medium from each group in each culture duration was pooled and assayed by the pigeon crop-gland method(1). Culture medium (0.05 ml) was injected intradermally for 4 days over the right crop gland and 1.25 μg lactogen† in 0.05 ml over the left crop gland of 9 to 10 pigeons per group. Lactogen content of 9-day cultured anterior pituitaries was estimated by injecting intradermally 1.25 mg/0.05 ml of tissue in an aqueous suspension over the right crop gland and 1.25 μg/0.05 ml lactogen over the left crop gland of 5 to 8 pigeons per group.

After lactogen assays, the medium in which anterior pituitaries of lactating rats were cultured 9 days was pooled into 4-day and 14-day lactating groups. Media from these two groups were dialyzed against distilled water for 18 to 24 hours and lyophilized. Protein components were separated electrophoretically on cellulose acetate in a tris buffer with an ionic strength of 0.05 and a pH of 8.8. Twenty-five μl of a solution containing 5 mg/ml of lyophilized medium were added per strip and electrophoretic separation was accomplished at 3°C for 5 hours with 150 volts and 0.70 milliamps per strip.

Representative AP fragments from each culture dish of all groups were examined histologically and degree of viability rated from zero to 4 as previously described(7). Differences between means were compared statistically using either Student's *t* test or analysis of variance for 2 variables(8).

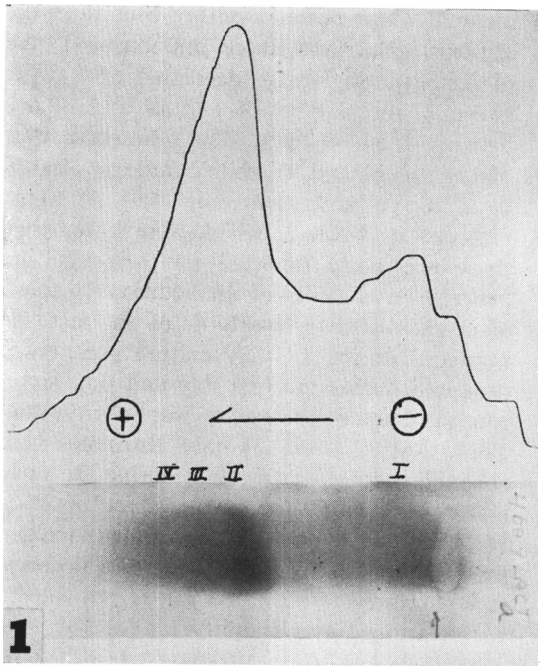
Results. In the separate experiments, lactogen production by anterior pituitaries of either 4- or 14-day lactating rats was not significantly different from that of AP of estrous cyclic rats for the 3 culture periods. When the data from all culture experiments were combined into 2 groups, lactating and estrous cyclic, and the effect of duration of culture statistically removed by an analysis of variance for 2 variables, lactogen production by AP from lactating rats was significantly greater than that of AP from estrous cyclic rats. When lactogen content in medium was adjusted for the greater pituitary weight of lactating animals (lactogen in medium/100

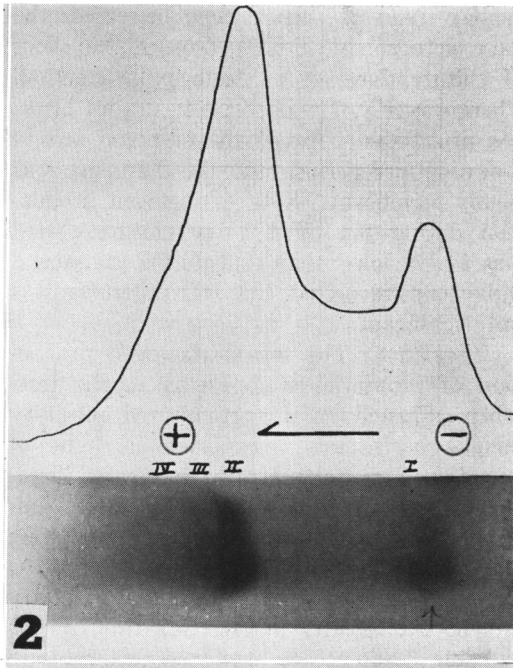
† The prolactin used in this work was a gift from the Endocrinology Study Section, N.I.H.

TABLE I. Lactogen Production by Anterior Pituitaries (AP) from Lactating Rats and Estrous Cyclic Rats During Prolonged Culture Periods.

Parameters	Exp No.*	Lactating AP	Estrous cyclic AP
	First 3 days		
Lactogen/100 ml medium (IU)†	1	45.1 ± 5.2	39.3 ± 3.3
	2	59.0 ± 3.9	48.1 ± 4.1
	Avg	52.0	43.7‡
Lactogen in medium /100 mg AP†	1	22.9 ± 2.6	24.2 ± 2.0
	2	31.5 ± 2.1	37.6 ± 3.2
	Avg	27.2	30.9‡
	Second 3 days		
Lactogen/100 ml medium (IU)†	1	34.7 ± 5.8	30.6 ± 3.4
	2	30.6 ± 3.6	28.6 ± 3.6
	Avg	32.7§	29.6‡§
Lactogen in medium /100 mg AP (IU)†	1	18.7 ± 3.1	20.1 ± 2.2
	2	17.0 ± 2.0	23.2 ± 2.9
	Avg	17.9§	21.7‡§
	Third 3 days		
Lactogen/100 ml medium (IU)†	1	30.1 ±3.1	27.5 ±6.3
	2	26.9 ±2.5	24.9 ±2.5
	Avg	28.5§‖	26.2§‖
Lactogen in medium /100 mg AP (IU)†	1	16.0 ±1.7	17.9 ±4.0
	2	14.4 ±1.4	19.6 ±2.0
	Avg	15.2§‖	18.8§‖¶
Avg AP wt post-cultured (mg)†**	1	5.45± .20	4.47± .25
	2	5.24± .22	3.58± .20
	Avg	5.35	4.03††
Lactogen in 100 mg cultured AP (IU)†	1	1.69± .38	1.54± .38
	2	1.75± .33	1.52± .63
	Avg	1.72	1.53‡
AP viability rating	1	1.75	1.75
	2	2.50	2.75
	Avg	2.13	2.25

* Exp 1: AP from lactating rats—avg 4 days; Exp 2: AP from lactating rats—avg 14 days.
† Mean and standard error of mean.
‡ Not significantly different ($P > .10$) from lactating AP.
§ Significantly lower ($P < .01$) than 3-day culture.
‖ Not significantly different ($P > .10$) from 6-day culture.
¶ Significantly higher ($P < .10$) than lactating AP.
** Ten AP cultured per group per experiment.
†† Significantly lower ($P < .01$) than lactating AP.





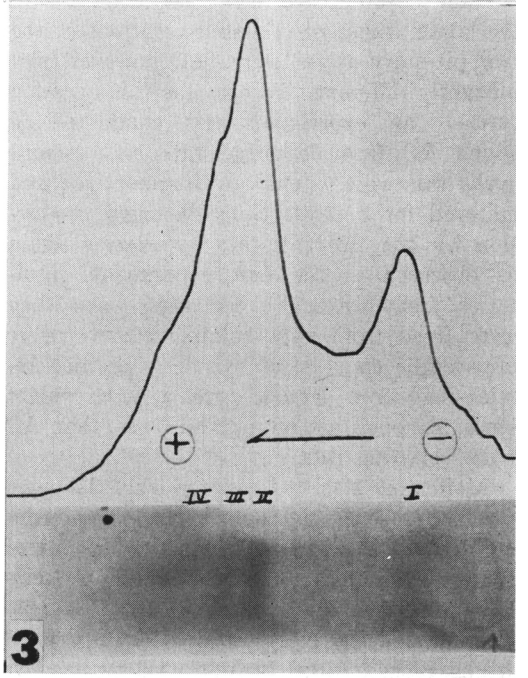


FIG. 1-3. Photographs of electrophoretic separation of lyophilized medium on cellulose acetate in a tris buffer with an ionic strength of 0.05 and a pH of 8.8. (1) Anterior pituitaries from rats lactating an average of 4 days. Pooled medium from first, second, and third 3 days of culture. (2) Anterior pituitaries from rats lactating an average of 14 days. Pooled medium from first, second, and third 3 days of culture. Note similarity of pattern to 4-day lactating group. (3) Anterior pituitaries from estrous cyclic rats. Medium from 3-day culture period. Note lower intensity of second component as compared to lactating group.

mg AP), pituitaries from estrous cyclic rats produced more lactogen than did glands from lactating rats. When the effect of duration of culture was statistically removed the difference was highly significant. Lactogen content, at the end of 9 days of culture, of AP from lactating animals did not differ significantly from that of AP from estrous cyclic animals and viability ratings were similar for both groups (Table I).

Lactogen production by pituitaries from rats lactating 4 days did not differ significantly from that by AP from rats lactating 14 days. The electrophoretic separation of lyophilized medium from these two groups resulted in similar patterns (Fig. 1, 2). Four protein components were present in the lyophilized medium and these components migrated in the electrical field in a manner similar to that noted for 3-day culture medium from AP of estrous cyclic rats (Fig 3). The only noticeable difference in this latter comparison was the greater intensity of the second component in the lactating groups.

Since lactogen production by AP from both lactating and estrous cyclic rats followed a

similar trend as culture time increased, the data were combined to determine the effect of culture duration on lactogen production. There was a 31.7% decrease in rate of lactogen production between the first and second 3-day culture periods and the difference was highly significant. Rate of lactogen production during the third 3-day culture period was 14.3% lower than that during the second 3-day culture period, but this difference was not significant.

Discussion. The greater lactogen production of pituitaries from estrous cyclic rats, when adjusted on a post-cultured pituitary weight basis, was unexpected in light of numerous reports indicating a greater lactogen concentration in AP from lactating animals. Since pituitaries from both lactating and estrous cyclic rats were divided into the same number of pieces, and since pituitary weight was greater in lactating rats, it was possible that necrosis was more extensive in the larger fragments. Viability ratings of the two pituitary types were not, however, significantly different. To examine this question further, an experiment was conducted in which AP from lactating rats and estrous cyclic rats were extensively fragmentized and cultured for 3 days. Since lactogen production by the two pituitary types was again as observed in the first experiment, pituitaries from estrous cyclic rats, once they were freed from hypothalamic control, were apparently capable of synthesizing and rereleasing more lactogen on a unit weight basis, although not on a gland basis, than AP from lactating rats.

Meites *et al*(6,9) reported that lactogen production could be detected by pituitaries cultured to 21 days, although the amount of tissue viable at that time was small. Pasteels (10) observed an increase in lactogen production during a culture period up to 3 weeks. Since natural medium used by Pasteels is known to induce tissue growth(11), the increase in total lactogen production with time may have been due to an increase in amount of AP tissue.

In our study the decrease in rate of lactogen production was greater in the earlier than in the later stages of culture. In an experiment in which optimal culture conditions did not exist (Gala and Reece, unpublished), rate of lactogen production decreased 65.7% between 3 and 6 days of culture and 39.6% between 6 and 9 days. The differences were highly significant. Viability ratings after 9 days of culture were only half of those recorded in Table I, which were lower than those previously reported for 3-day culture periods(7). Results of an additional experiment showed that two-thirds of the lactogen produced during a 3-day culture period was produced during the first day and that lactogen production thereafter was nearly constant. Ratner *et al*(12) have also noted considerable lactogen production during the early phase of culture. Considering these observations, it appears that the decrease in lactogen production with time is not due to a decrease in lactogen secretion rate *per se* but due to a decrease in tissue viability.

Summary. Anterior pituitaries from lactating and from estrous cyclic hooded Norway rats were cultured simultaneously for 9 days. Lactogen production by pituitaries from lactating rats was slightly greater than that by glands from estrous cyclic rats, but when lactogen production was expressed on a unit weight basis it was significantly greater by pituitaries from estrous cyclic rats. Electrophoretic separation of medium from pituitaries of lactating rats revealed patterns similar to those obtained from medium from pituitaries of estrous cyclic rats. Rate of lactogen production decreased 31.7% from the first 3-day culture period to the second 3-day culture period, and 14.3% from the second 3-day to the third 3-day culture period. Evidence was presented which indicated that the decrease in lactogen production with time was due not to a decrease in production rate *per se* but rather to an increase in anterior pituitary tissue necrosis.

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Mucopolysaccharide Production By Fibroblasts Derived from Human Buffy-Coat Cultures *in vitro*.* (29328)

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Grossfeld(1) has shown that fibroblasts from different tissues cultivated *in vitro* are able to produce acid mucopolysaccharides (AMPS) and release them into the medium. AMPS belong to the group of hyaluronic acid and chondroitin sulphates A and C, are highly polymerized, and give positive mucin clot and hyaluronidase tests(2).

Since fibroblast-like cells are known to originate in cultures of buffy-coat and hemapoietic tissues(3,4), it seems of interest to study the AMPS production in human white blood cell cultures undergoing fibroblastic transformation.

Materials and methods. Peripheral human blood buffy-coat was obtained by venipuncture and centrifugation at 600 × *g* with 0.1 mg/ml of heparin (Liquemine Roche) for each 5 ml of blood and phytoagglutinine (Difco), 0.25 ml for each 10 ml of blood. The first 1 or 2 ml extracted from the vein was discarded to avoid contamination with tissue cells. Buffy-coats from 10 ml of blood were explanted in Earle's flasks with a layer of isologous plasma and 5 ml of liquid medium containing yeast extract-Eagle medium-lactalbumin hydrolysate-peptone(5) and 5% chicken embryo extract.

Turbidimetric tests of hyaluronidase according to Seastone and Kass(6) were performed as previously described(7) in samples of culture media taken every 4 days. The medium was centrifuged to eliminate cells and debris that might interfere with the turbidimetric tests. Incubated medium without explants was used as a blank. Determinations were also made on aliquots of homogenized cells before and after cultivation. The cells were homogenized in phosphate buffer pH 6.5 (0.1 M) with Potter-Elvehjem homogenizer and centrifuged at 800 × *g*. The supernatant was taken for analysis.

Hyaluronidase from Benger Lab. Lt. was used (Hyalase Benger). It was found that 500 U Benger was the optimum necessary to reduce turbidity to 50%. Standard curves were made with pure hyaluronic acid prepared at Prof. Karl Meyer's Laboratory.

Results. a) *Appearance of fibroblast-like cells.* Fibroblast-like cells start to appear about 5 days after explantation (Fig. 1). Some of the cells have the characteristic appearance of macrophages and others have distinct fibroblast features. By the second week of cultivation, a thick layer of fibroblasts can be observed. b) *Release of AMPS in the medium.* Estimation of AMPS in the first change of medium (4 days) shows a high content, amounting to about 50% of the total AMPS of the explanted cells. This may be due to release of AMPS from degenerating granulocytes, platelets and cell debris which are found in suspension in the liquid medium. Thereafter, release of AMPS into the medium

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