ited the incorporation of acetate-1-C^{14} into sterols in intact mice even after a single injection. Prolonged treatment of mice with very low, subconvulsive, doses of the drug resulted in a 20% decrease in plasma cholesterol level, as well as a pronounced decrease in the amount of radioactivity found in liver sterols synthesized from acetate-1-C^{14} or mevalonate-2-C^{14} but not from squalene-C^{14} or lanosterol-T. Metrazol treatment also resulted in almost complete absence of radioactivity in carbon dioxide exhaled by treated animals injected with mevalonate-2-C^{14}. These findings indicate that the site of action of Metrazol along the acetate-cholesterol pathway lies between mevalonate and squalene.

We wish to express our gratitude to Dr. Warren M. Sperry, without whose assistance and encouragement this work would not have been possible.

1. Alexander, G. J., Alexander, R. B., *Biochemistry,* 1962, v1, 783.

2. Alexander, R. B., Alexander, G. J., *Abstr. Comm., 5th Intern. Congress Biochem.,* Moscow, 1961, p533.

3. Alexander, G. J., Alexander, R. B., PROC. SOC. EXP. BIOL. AND MED., 1962, v107, 352.

4. Sperry, W. M., Webb, M., *J. Biol. Chem.,* 1950, v187, 97.

5. Schwenk, E., Alexander, G. J., Fish, C. A., *Arch. Biochem. Biophys.,* 1955, v58, 37.

6. Schwenk, E., Werthessen, N. T., *ibid.,* 1952, v40, 334.

7. Lindberg, M., Gautschi, F., Bloch, K., *J. Biol. Chem.,* 1963, v238, 1661.

8. Steinberg, D., *Trans. N. Y. Acad. Sci.,* II, 1962, v24, 704.

9. Siperstein, M. D., Guest, M. J., *J. Clin. Invest.,* 1960, 439, 642.

10. Cook, R. P., *Cholesterol,* Academic Press, 1958, p160.

11. Korey, S. R., Stein, A., in *Regional Neurochemistry,* eds. Kety, S. S., Elkes, J., Pergamon Press, 1961, p175.

12. Sperry, W. M., *Mental Retardation,* 1962, v39, 47.

Received October 9, 1963. P.S.E.B.M., 1964, v115.

Influence of Sodium Bicarbonate Level and Culture Temperature on Anterior Pituitary Lactogen Production *in vitro*.* (28878)

RICHARD R. GALA AND RALPH P. REECE

Department of Dairy Science, New Jersey Agricultural Experiment Station, New Brunswick

Various investigators(1,2,3) have cultured anterior pituitaries (AP) *in vitro* and obtained the production of lactogen in excess of the amount initially introduced with the tissue. Pasteels(2), using a natural medium, did not indicate the culture temperature, and Meites and co-workers(2,3), using a synthetic medium, cultured AP from various species at 35°C. In subsequent studies Meites and co-workers(4,5) cultured pituitaries at a temperature of 37°C; in these experiments, however, the culture duration was for a shorter time and no comparison could be made with the initial reports. In all the studies using a synthetic medium(1,3,4,5), bicarbonate was added to the medium at a constant level. This investigation was conducted to determine the influence of level of sodium bicarbonate and culture temperature on AP lactogen production *in vitro*.

Materials and methods. Anterior pituitaries were obtained from female, hooded Norway rats approximately 3 months of age. In Experiments 5, 6, 7, and 8 of the 2.0 ml bicarbonate group, pituitaries were from animals weighing 180-200 g, while in all other experiments, pituitaries were from animals weighing 165-175 g. Culture techniques were similar to those reported by other investigators(1). Anterior pituitaries were divided into 6 pieces and one AP was placed in each culture dish. Six to 10 dishes were cultured per experiment per group for a period of 3

* Paper of the Journal Series, N. J. Agric. Exp. Station, Rutgers—The State University, New Brunswick.

days. In the bicarbonate study, either 2.0 or 2.5 ml of 2.8% $NaHCO_3$ were added per 25 ml of medium, and AP were cultured at 35°C. In the temperature study, 2.0 ml of 2.8% $NaHCO_3$ were added per 25 ml of medium and AP were cultured at either 35°, 37°, or 39°C. In Experiments 6 and 7 of the 2.5 ml bicarbonate group insulin† was added to the medium at a concentration of 0.1 mg/ml; in all other experiments, no insulin was added to the medium. The antibiotics, streptomycin and penicillin, were added to the medium at a level of 0.2 mg and 0.068 mg/ml medium, respectively. The gas environment consisted of 95% O_2 – 5% CO_2 at a flow rate of either 150 or 300 cc/min.

The lactogen content in medium and cultured tissue was determined by the crop gland proliferation method of Reece and Turner (6). Pooled medium from each experiment was injected intradermally over the right crop gland of mature pigeons at a level of 0.05 ml/day and 1.25 μg lactogen‡ (0.01875 IU), in the same volume, was injected over the left crop gland for 4 days. Pigeons were sacrificed on the fifth day and degree of proliferation rated visually. Cultured AP tissue was assayed by injecting an aqueous suspension of 1.25 mg AP/0.05 ml/day over the right crop gland and the lactogen standard over the left crop gland. Lactogen content in the medium was estimated using 6 to 10 pigeons per experiment; 2 to 4 pigeons were used to estimate the lactogen content of cultured AP tissue per experiment.

Representative anterior pituitary pieces from each experiment were examined histologically and estimates of the degree of viable tissue rated as previously described(7). Differences between means were compared statistically using Student's *t* test.

Results. In the bicarbonate study, a total of 8 and 9 experiments was run with 2.0 and 2.5 ml of 2.8% $NaHCO_3$ added per 25 ml of medium, respectively. Anterior pituitaries in the 2.0 ml group were heavier than those in the 2.5 ml group. This was believed to be due to some AP obtained from animals that were slightly heavier. When adjusted for the difference in pituitary weight between the two groups, lactogen production by AP cultured in the 2.0 ml group was 19.2% higher than that in the 2.5 ml group. Lactogen content of cultured AP tissue and pituitary viability ratings for the 2.0 ml group were, however, significantly lower than those for the 2.5 ml group (Table I).

A total of 12 experiments was conducted to determine the effect of culture temperature on lactogen production; 5 at 35°C, 5 at 37°C, and 2 at 39°C. Lactogen production by AP cultured at 37°C was 22.5% higher than AP cultured at 35°C, and 16.7% higher than AP cultured at 39°C. Viability ratings for pituitaries cultured at 35° and 37°C were similar, but pituitaries cultured at 39°C had significantly lower viability ratings than those cultured at either 35° or 37°C. Lactogen content of pituitaries cultured at 37°C was similar to that for pituitaries cultured at 35°C (Table II).

Discussion. The net synthesis of lactogen during a 3-day culture period has been reported to be 6 to 9 times greater than that initially introduced into the system(1,3). Meites and co-workers(8,9) reported that they had improved the culture technique and now obtained a 30-fold increase in lactogen production during the 3-day culture period. They did not state, however, what improvements were made. We have reported previously(7) a 6- to 8-fold net synthesis in pituitary lactogen production *in vitro*. By adjusting the bicarbonate present in the medium, we have now been able to obtain almost 9 times more lactogen than that initially introduced with the pituitary. Additional culture temperature studies revealed that AP cultured at 37°C were capable of synthesizing and releasing into the medium 11.2 times more lactogen than that which was present initially.

In the bicarbonate study, the greater lactogen production by AP in the 2.0 ml group was even more convincing when one considers

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

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

TABLE I. Influence of Level of 2.8% $NaHCO_3$ Added per 25 ml of Medium on Anterior Pituitaries Cultured *in vitro*.

Level of 2.8% $NaHCO_3$/25 ml medium	Exp No.	Avg AP wt post-culture (mg)*†	Lactogen/ 100 ml medium (IU)*	Lactogen in medium /100 mg AP (IU)*	Lactogen in 100 mg cultured AP (IU)*	AP viability rating
2.0 ml	1	3.73 ± .39	38.9 ± 6.3	28.1 ± 4.6	—	3.00
	2	4.17 ± .33	42.9 ± 3.0	27.6 ± 1.9	1.00 ± .45	3.00
	3	4.03 ± .21	48.0 ± 7.9	32.3 ± 5.7	1.40 ± .36	2.75
	4	4.13 ± .14	34.6 ± 7.8	23.3 ± 5.2	1.44 ± .30	2.50
	5	5.07 ± .30§	32.9 ± 2.8	17.8 ± 1.5	—	1.50
	6	4.85 ± .31§	43.8 ± 6.3	25.3 ± 3.6	—	1.75
	7	4.43 ± .14§	46.2 ± 6.8	28.5 ± 4.9	—	1.50
	8	4.63 ± .34§	35.3 ± 4.8	21.2 ± 2.9	—	1.50
	Avg	4.38	40.3	25.5¶	1.28**	2.19**
2.5 ml	1	4.22 ± .12	28.9 ± 3.3	19.2 ± 2.2	1.88 ±1.10	3.50
	2	3.92 ± .23	31.9 ± 4.2	22.5 ± 3.0	—	3.00
	3	3.35 ± .26	23.6 ± 3.6	19.2 ± 2.9	—	3.00
	4	3.95 ± .13	21.0 ± 2.3	15.2 ± 1.6	—	3.50
	5	4.10 ± .21	25.1 ± 4.1	17.6 ± 2.8	—	3.25
	6‖	3.60 ± .27	29.5 ± 7.9	21.1 ± 5.6	1.02 ± .26	2.50
	7‖	4.34 ± .35	48.4 ± 3.9	30.4 ± 2.5	2.33 ± .54	3.00
	8	3.98 ± .10	36.0 ± 7.3	24.6 ± 5.0	2.50 ± .35	2.50
	9	4.15 ± .30	35.0 ± 4.3	22.6 ± 1.6	2.25 ±0 ‡	2.50
	Avg	3.96	31.0	21.4	1.99	2.97

* Mean and standard error of mean.
† Six AP cultured per experiment.
‡ All pigeons (2) gave the same response.
§ AP taken from larger animals (180-200 g) than usual (165-175 g).
‖ Zinc-free insulin added at level of .1 mg/ml medium.
¶ Significantly higher ($P < .05$) than 2.5 ml group.
** Significantly lower ($P < .05$) than 2.5 ml group.

TABLE II. Influence of Temperature on Anterior Pituitaries Cultured *in vitro*.

Temperature	Exp No.	Avg AP wt post-culture (mg)*†	Lactogen/ 100 ml medium (IU)*	Lactogen in medium /100 mg AP (IU)*	Lactogen in 100 mg cultured AP (IU)*	AP viability rating
35°C	1	3.73 ± .39	38.9 ± 6.3	28.1 ± 4.6	—	3.00
	2	4.03 ± .21	48.0 ± 7.9	32.3 ± 5.7	1.40 ± .36	2.75
	3	4.13 ± .14	34.6 ± 7.8	23.3 ± 5.2	1.44 ± .30	2.50
	4	4.48 ± .25	26.3 ± 4.0	16.2 ± 2.5	2.83 ± .50	3.00
	5	4.17 ± .33	42.9 ± 3.0	27.6 ± 1.9	1.00 ± .45	3.00
	Avg	4.11‡	38.1	25.5**	1.67§	2.85§
37°C	1	4.47 ± .20	61.3 ± 4.4	38.1 ± 2.7	2.05 ± .22	3.25
	2	4.13 ± .20	49.8 ± 5.5	32.1 ± 4.0	2.25 ± .75	3.00
	3	4.90 ± .16	65.3 ± 6.3	35.1 ± 3.4	—	3.00
	4	3.71 ± .21	43.0 ± 4.8	29.7 ± 3.3	—	2.50
	5	4.04 ± .18	44.0 ± 6.1	29.6 ± 4.1	—	3.00
	Avg	4.25	52.7	32.9	2.15	2.95
39°C	1	4.28 ± .14	42.6 ± 7.9	26.3 ± 4.9	—	2.25
	2	4.11 ± .13	51.1 ± 7.7	30.0 ± 4.5	—	2.50
	Avg	4.20‡	46.9	28.2§‖	—	2.33¶††

* Mean and standard error of mean.
† Six to ten AP cultured per experiment.
‡ Not significantly different ($P > .10$) from each other.
§ Not significantly different ($P > .10$) from 37°C.
‖ Not significantly different ($P > .10$) from 35°C.
¶ Significantly lower ($P < .10$) than 37°C.
** Significantly lower ($P < .05$) than 37°C.
†† Significantly lower ($P < .10$) than 35°C.

that anterior pituitary viability for this group was significantly less than that for the 2.5 ml group. The lower viability rating of the 2.0 ml group was believed to be due to the culturing of larger pituitaries in some of the experiments. These larger pituitaries when examined histologically had an area of central necrosis that could be attributed to the lack of nutrients and/or gas environment in this area. It was necessary, therefore, that pituitaries be adequately fragmentized to allow for proper nutritional and gaseous exchange to all cells.

Addition of insulin to the medium in some of the experiments in the bicarbonate study appeared to have no effect on lactogen production. This observation was in accord with reports of other workers(10).

In the temperature study lactogen production increased with increasing temperature to 39°C, when it decreased below that noted for 37°C. This decrease was believed to be due to tissue damage, since AP viability ratings were significantly lower.

Summary. The influence of level of sodium bicarbonate and temperature on lactogen production by rat anterior pituitaries cultured *in vitro* was studied. Pituitaries were cultured for 3 days in the chemically defined mixture 199. When compared with the 2.5 ml group, addition of 2.0 ml of 2.8% $NaHCO_3$ per 25 ml medium resulted in a 19.2% higher lactogen production. Anterior pituitaries cultured at 37°C produced 22.5% more lactogen than those cultured at 35°C, and 16.7% more than those cultured at 39°C. At the highest temperature, AP tissue damage was noted. When the bicarbonate level and culture temperature were optimum for lactogen production, anterior pituitaries synthesized and released 11.2 times more lactogen than that which was initially introduced into the culture system with the pituitary gland.

1. Meites, J., Kahn, R. H., Nicoll, C. S., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 440.
2. Pasteels, J. L., *Compt. rend. Acad. Sci.,* 1961, v253, 2140.
3. Nicoll, C. S., Meites, J., *Nature,* 1962, v195, 606.
4. Ratner, A., Talwalker, P. K., Meites, J., *Fed. Proc.,* 1962, v21, 195.
5. Meites, J., Talwalker, P. K., Ratner, A., *Proc. 44th Meeting Endocrine Soc.,* 1962, p19.
6. Reece, R. P., Turner, C. W., *Mo. Agric. Exp. Sta. Res. Bull.,* 1937, 266.
7. Gala, R. R., Reece, R. P., PROC. SOC. EXP. BIOL. AND MED., 1963, v114, 422.
8. Nicoll, C. S., Meites, J., *Endocrinology,* 1962, v70, 272.
9. ———, *ibid.,* 1962, v70, 927.
10. ———, *ibid.,* 1963, v72, 544.

Received October 9, 1963. P.S.E.B.M., 1964, v115.

Possible Role of Leucocyte Granules in the Shwartzman and Arthus Reactions.* (28879)

LEWIS THOMAS
Department of Medicine, New York University School of Medicine, and Third and Fourth (N.Y.U.) Medical Divisions, Bellevue Hospital

Polymorphonuclear leucocytes appear to play an essential role in the pathogenesis of the Shwartzman and Arthus reactions. Both are characterized by extensive infiltration of the involved tissues by these cells prior to the development of hemorrhage and necrosis, and both can be prevented by agents causing polymorphonuclear leucopenia(1-3). In the Arthus reaction, Cochrane and Weigle(4) have shown that temporary leucopenia does not prevent the formation of antigen-antibody complexes in the skin, but tissue destruction begins to occur only when leucocytes appear in the area.

* Work performed during sabbatical leave sponsored by a fellowship award of the Commonwealth Fund. The studies were supported, in part, by a grant from Nat. Inst. Health, and under a contract with Armed Forces Epidemiological Board, Office of Surgeon General.