conclusion from experiments on conductivity, that in mammalian nerves *in vivo* asphyxia affects the large fibers first.

8717 C

Effect of Lactogenic Hormone on Embryonic Tissues Cultivated *in vitro*.

A. J. SALLE AND I. L. SHECHMEISTER.

From the Department of Bacteriology, University of California, Berkeley.

Since lactogenic hormone* is known to produce a specific effect on epithelial cells of the pigeon crop[1] *in vivo,* experiments were designed to determine whether it would stimulate the growth of embryonic pigeon crop epithelium cultivated *in vitro.* If stimulation could be observed in this manner such a test might be more delicate and superior to the present methods employed for determining hormonal potency.

Preliminary to testing the hormone on the specific crop epithelium, which reacts to it *in vivo,* experiments were carried out on the following non-specific tissues cultivated *in vitro:* (1) Embryonic chick connective tissue, (2) embryonic chick epidermis, (3) embryonic pigeon oesophageal epithelium. Finally the hormone was tested on cultures of embryonic epithelium taken from the crop sac areas known to be reactive *in vivo* in the hatched pigeons.

In all cases the dilutions were performed in triplicate. Also, every experiment was repeated 3 times before the results were reported. Each flask contained about 12 fragments of tissue.

(1) *Embryonic Chick Connective Tissue.* Chick heart tissue from 10- to 14-day-old embryos was used as the source of fibroblasts. The tissues were finely cut into fragments of about 1 mm. in diameter and immersed in Tyrode solution until ready for use.

The embryonic fluid was prepared by mincing 14-day-old chick embryos in a tissue grinder and suspending the pulp in 4 parts of

* Anterior pituitary mammotropic principle, prolactin, galactin, mammotropin. The preparations used in these experiments were kindly supplied by Dr. W. R. Lyons of the Division of Anatomy. The hormone caused crop sac epithelial growth in 24-48 hours when given in doses as low as 0.1 microgram intradermally over the crop sac area.

1 Riddle, O., Bates, R. W., and Dykeshorn, S. W., *Am. J. Physiol.,* 1933, **105**, 191.

Tyrode solution. The suspension was centrifugated at high speed to throw down all cellular material after which the clear supernatant fluid was carefully removed with a pipette. This clear liquid was used for coagulating the plasma as well as a nutrient for the tissue cells.

Guinea pig plasma was used in all experiments. This was collected by first drawing 1.0 cc. of a 1-1,000 solution of heparin into a 10.0 cc. syringe and then heart blood to the 10.0 cc. mark. The mixture was quickly centrifugated, the clear plasma removed with a pipette and kept in ice water until ready for use.

The hormone preparation† was added to the solid phase only. The various components and their proportions are summarized in Table I.

TABLE I.

Components	No. of drops used					
	Hormone-Treated Flasks					Control Flasks
Plasma	3	3	3	3	3	3
Tyrode Solution	4	3	2	1	0	5
Embryonic fluid	2	2	2	2	2	2
Hormone Solution	1	2	3	4	5	0
Total No. of drops	10	10	10	10	10	10

The various mixtures were prepared in Carrel flasks. Ten drops of embryonic fluid were added after coagulation of the plasma. This furnished the nutrients for the tissue cells.

The growth in all flasks, both hormone-treated and non-hormone treated was of the same order after an incubation period of 48 hours.

(2) *Embryonic Chick Epidermis.* Skin tissue from 7, 9, 10, and 12-day-old chick embryos was used as the source of epithelial cells. Mixed growth of fibroblasts and epithelial cells occurred in many of the fragments but the fibroblastic growth was disregarded.

In the first series of experiments graduated amounts of hormone was added to the embryonic fluid following coagulation of the plasma. No hormone was present in the solid phase. Epithelial stimulation was negative after an incubation period of 72 hours.

In the following series of experiments the hormone was added to the solid phase only. The set-up is given in Table II.

Ten drops of embryonic fluid was added following coagulation of the plasma.

In another series of experiments the same set-up as given above

† The hormone preparation contained 1 gm. of organic solids in 100 cc. of solution. This is, for convenience, called a 1% solution.

TABLE II.

Components	No. of drops used				
	Hormone-Treated Flasks				Control Flasks
Plasma	3	3	3	3	3
Tyrode Solution	3	2	1	0	5
Embryonic Fluid	4	4	4	4	4
Hormone Solution	2	3	4	5	0
Total No. of drops	12	12	12	12	12

was employed except that hormone was added to both the solid and liquid phases. Consistently negative stimulation was obtained in all experiments.

(3) *Embryonic Pigeon Oesophageal Epithelium.* In the experiments to follow embryonic pigeon oesophageal epithelium was employed. The oesophagus in the region of the crop sac was removed aseptically from a pigeon just before hatching. The components of the cultures and their proportions were the same as given in Table II.

In the first series of experiments hormone was added to the solid phase only. In the second series hormone was added to the liquid phase only. Tyrode solution was substituted for the hormone in the solid phase. In the final series hormone was added to both solid and liquid phases. Growth stimulation was negative in all experiments after an incubation period of 48 hours.

(4) *Embryonic Pigeon Crop Epithelium.* Tissue from the embryonic crop gland of a pigeon was used. The glandular tissue was removed aseptically and reduced to fragments as uniform in size as possible.

The components and proportions of each were the same as given in Table II. The following series of experiments were performed:

1. Tissue fragments bathed in Tyrode solution previous to embedding. Hormone solution was added to (a) liquid phase only, (b) solid phase only, and (c) both solid and liquid phases.

2. Tissue fragments bathed in hormone solution previous to embedding. Hormone solution added to (a) liquid phase only, (b) solid phase only, and (c) both liquid and solid phases.

(3). Tissue fragments were embedded and allowed to incubate for 36 hours before adding hormone to the liquid phase. Five dilutions of hormone were used ranging from 1:10 to 1:100,000.

Measurements of the areas of the tissue fragments were made and percentage increases recorded. A total of 220 measurements on 93 tissue fragments were made. The figures showed no growth stimulation of the fragments in the presence of hormone.

It may be concluded that lactogenic hormone, although capable of producing a specific effect on epithelial cells of the immature pigeon crop *in vivo*, is unable to stimulate embryonic crop tissue when cultivated *in vitro*. Non-specific tissues such as embryonic chick connective tissue, embryonic chick epidermis and embryonic pigeon oesophageal epithelium also failed to show growth stimulation in the presence of the hormone.

8718 P

Effect of Autoclaved Pancreas upon Lipids of Blood and Liver in Depancreatized Dogs Maintained with Insulin.*

A. KAPLAN AND I. L. CHAIKOFF.

From the Division of Physiology, University of California Medical School, Berkeley.

It was first shown in this laboratory that raw pancreas contains a factor (or factors) active on the blood lipids of the completely depancreatized dog maintained with insulin.[1] When the glandular tissue was added to the diet immediately after pancreatectomy, a rise above normal in blood lipid constituents occurred. The removal of the glandular tissue from the diet resulted in an abrupt fall in the lipid constituents, in particular, cholesterol esters. The essential effect of pancreas feeding upon blood lipids consisted in the maintenance of a level, particularly cholesterol, above the preoperative or normal; this effect was produced irrespective of whether the concentration of the blood lipids was normal or subnormal at the time when feeding of the glandular tissue was instituted.

It has already been shown that the blood lipid factor is not choline.[2] In a further study of the mechanism whereby raw pancreas produces these lipid changes, it seemed of interest to investigate the effects of pancreas that had been autoclaved for 30 minutes at 20 lb. pressure. Such a procedure destroys the enzymes but leaves unaltered the caloric value of the ingested pancreas.

A typical result of the effect of autoclaved pancreas on the blood

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1 Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1935, **112**, 155.

2 Chaikoff, I. L., and Kaplan, A., PROC. SOC. EXP. BIOL. AND MED., in press.