Effect of Amniotic Fluid on Cortisone Inhibition of Growth. (22049)

PHILIP O'BRYAN MONTGOMERY.

Department of Pathology, University of Texas Southwestern Medical School, Dallas.

Normal growth under certain conditions is inhibited by systemic cortisone. One outstanding example of this biologic property of cortisone is its ability to inhibit normal wound healing(1,2). The local applications of beef amniotic fluid have been observed to reverse completely this systemic action of cortisone on healing wounds(3). Accordingly it seemed advisable to determine the effect of beef amniotic fluid upon other growing cellular systems. For this purpose the newly hatched chick was selected as a model.

Methods. Newly hatched White Rock chicks, obtained from a local hatchery, were fed Purina chick starter and water *ad lib.*, while being kept in a chick brooder incubator in the laboratory. The chicks were divided into the following groups: (a) 37 chicks were allowed to grow normally for 18 days; (b) 36 chicks received 5 mg of Cortisone (Merck & Co.) subcutaneously daily for 18 days;† (c) 27 chicks received daily subcutaneous injections of beef amniotic fluid for 18 days. The dose was increased from 5 cc to 10 cc per day as the chicks grew; (d) 35 chicks received 5 mg of cortisone in addition to 5 to 10 cc of beef amniotic fluid per day for 18 days. All chicks were weighed at the beginning of the experiment and at 18 days. Amniotic fluid was not given on the 18th day.

Results. The average weight gain of each chick per group is illustrated in Table I. Fig. 1 shows a photograph of three 18-day-old chicks. The chick on the left received only cortisone. The chick in the middle received cortisone plus beef amniotic fluid. The chick on the right is a normal 18-day-old chick. This study indicates that the chicks receiving beef amniotic fluid in addition to cortisone are larger and better developed than their mates receiving cortisone alone; although not the equal of their normal mates. It is of interest to note that beef amniotic fluid administered alone did not promote or accentuate normal growth. In fact, the chicks of group (c) show less average weight gain than normal.

TABLE I.

Group	No. of chicks	Avg wt gain in 0-18 days (g)
A. Normal growth	37	136.2
B. Cortisone treated	36	28.9
C. Amniotic fluid treated	27	91.3
D. Cortisone & amniotic fluid	35	49.9

* Supported in part by U.S.P.H.S. through the National Advisory Arthritis and Metabolic Diseases Council.

† The Cortisone was kindly furnished by Merck & Co.

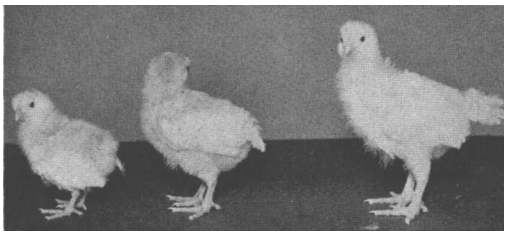

FIG. 1. Three 18-day-old chicks. Left chick given 5 mg cortisone daily, middle chick given cortisone plus amniotic fluid, right chick represents normal growth.

Beef amniotic fluid is known to contain growth-promoting and stimulating properties and is a satisfactory media for the tissue culture of mammalian cells. *In vitro* this may be the result of a ready supply of preformed nucleo-proteins(4-6).

With the possibility in mind that liver nucleo-protein may have been modified, liver sections from 8 chicks of groups (a), (b), and (d) were analyzed for their ribonucleoprotein content. The standard technic of toluidine blue staining before and after ribonuclease digestion of comparable sections was used(7). No differences in the liver ribonucleoprotein content were found by this technic in the livers from the 3 groups studied. It would seem unlikely that the liver content of nucleoprotein was effected by the procedures employed in the 3 groups studied.

Summary. Cortisone systemically administered to day-old chicks is a powerful growth

inhibitor for the 18-day period of this study. A partial reversal of this growth inhibitory activity of cortisone may be accomplished by the simultaneous administration of beef amniotic fluid.

I wish to thank Ovia Walker, Angela Ramos, and Tom Dillon for their technical assistance.

1. Howes, E. L., *Surgery,* 1950, v28, 177.
2. Spain, D. M., and Malomut, M., *Science,* 1950, v112, 335.
3. Montgomery, P., O'B., and Green, C., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 657.
4. Fischer, A., *Nature,* 1930, v144, 113.
5. ———, *Chem. Products,* 1940, v3, 79.
6. ———, *Acta Physical Scand.,* 1942, v4, 207.
7. Herman, E., *Histochemical and Cytological Techniques,* 22nd edition, Department of Anatomy, Harvard Medical School.

Received September 12, 1955. P.S.E.B.M., 1955, v90.

Effect of Temperature Variations on Paper Electrophoretic Migration of Serum Lipoproteins.* (22050)

J. C. FORBES AND P. C. TAYLOR.
Department of Biochemistry, Medical College of Virginia, Richmond.

In a study on filter paper electrophoresis of serum proteins it became quite obvious that temperature changes markedly affected the migration of the serum lipoproteins even though the migration of the main serum proteins was affected to only a negligible extent. In order to determine what variations extremes in laboratory temperature might cause, we have carried out a study on the cholesterol distribution along filter paper strips following electrophoresis at 5°C versus summer room temperature and 35°C. The summer room temperature, though not known exactly, was probably around 26°C to 30°C most of the time.

Methods. A Kelab paper electrophoresis apparatus was used with Whatman #3 filter paper strips (4.6 x 35 cm) and a barbital-sodium acetate buffer of pH 8.6 and ionic strength .05. Three strips of filter paper separated from one another by plastic strips were placed between the glass plates of each of the 2 compartments, thus making a total of 6 strips for each run. To each of the strips at a line 12 cm from the cathode end of the strip a total of 0.05 ml of serum was applied evenly across the strip except for a margin of 2 mm at each edge. The duration of the electrophoresis was 15 hours. A current of 1.2 milli-amps per strip was employed consistently and the potential was approximately 210V. At the end of the electrophoretic procedure all strips were removed and processed as follows: a) 2 strips were oven dried at 100°C for about 45 minutes then stained with bromphenol blue for protein after the method of Köiw *et al.*(1); b) one strip was air dried then stained with Sudan Black-B for lipids; and c) the other 3 strips were kept from drying by suspending them in a 1000 ml glass cylinder containing water at the bottom and covered with an inverted petri dish to reduce evaporation. As soon as the position of the various proteins along the dried strips was determined, which seldom exceeded 90 minutes from the time the strips were removed from the electrophoretic apparatus, the wet strips were cut as indicated below and the sections transferred to 25 ml glass stoppered Erlenmeyer flasks. Nine sections were employed: 1) the albumin area, which also contained the alpha$_1$-globulin; 2) the area between the albumin, alpha$_1$-globulin and the alpha$_2$-globulin; 3) the alpha$_2$-globulin; 4) a narrow strip between the alpha$_2$- and beta-globulins; 5) the beta-globulin; 6) a strip between the beta-globulin and 5 mm from the starting point; 7) the area from 5 mm of the starting point to 5 mm on the other side of the starting point

* Grateful acknowledgement is made to the Reynolds Metals Co. for research grant and to Lederle Laboratories for summer research fellowship to PCT.