14. Karmen, A., *J. Clin. Invest.*, 1955, v34, 126.
15. Ordell, R., *Opusc. Med.*, 1956, v1, 16.
16. Molander, D. W., Wróblewski, F., LaDue, J. S., *J. Lab. Clin. Med.*, 1955, v46, 831.
17. Wróblewski, F., LaDue, J. S., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 569.
18. D'Amour, F. E., Blood, F. R., *Manual for Laboratory Work in Mammalian Physiol.*, 1954.
19. Kato, N., Murakami, S., *J. Lab. Clin. Med.*, 1959, v54, 365.

Received April 19, 1961. P.S.E.B.M., 1962, v109.

Effect of Cortisone and Growth Hormones on Cellular Proliferation During Early Embryogenesis.* (27172)

M. M. SAYEED, D. S. BLUMENTHAL AND H. T. BLUMENTHAL
Institute of Experimental Pathology, The Jewish Hospital, St. Louis, Mo.

A number of studies have dealt with the effects of cortisone acetate and pituitary growth hormone on embryonal tissue(1-7). In chick embryos the growth-retarding effect of cortisone is well known(1,5,6,7). It has been reported that this effect is demonstrable as early as the fourth day of embryogenesis (5). Such an early retardation of growth is manifested in an alteration of the average length of the embryos and in a suppression of mitotic activity. Growth hormone administered to chick embryos during the last half of embryogenesis results in a striking increase in size and in mitotic activity of the tibia, increase in body weight, elevation in the blood sugar of newly hatched chicks and in increased nucleic acid synthesis(2,3,4). Effects of growth hormone during early embryogenesis appear not to have been reported heretofore.

The present report compares the effects of cortisone and growth hormone during an even earlier period of embryogenesis (18-72 hours). While it has already been shown that cortisone can exert an effect on the chick embryo before development of its own functional endocrine system, it is not yet known if growth hormone can also be effective during this period.

Materials and method. Fertile white New Hampshire chick eggs were incubated at a temperature between 98° and 98.5°F and 80% relative humidity. Suspensions of cortisone acetate (Merck Sharp and Dohme) and beef growth hormone (Armour) were prepared in sterile normal saline. Twenty dozen eggs were divided into 4 equal groups, which were injected chorioallantoically on initial day of incubation. One group received 1 mg of cortisone in 0.2 ml of saline and a second group 20 μg of growth hormone in 0.2 ml of saline. One control group received 20 μg of albumin in 0.2 ml of saline and a second 0.2 ml of saline only. Because of the high mortality in the younger embryos receiving albumin, it was used only in the 72-hour experiment.

Embryos were removed after 18, 30, 60 and 72 hours of incubation, fixed in Bouin's fluid, stained *in toto* with hematoxylin, embedded in paraffin and serially sectioned at 12 μ. All mitotic figures were counted in 10 random sections on each slide under oil immersion. This was done on ectodermal, mesodermal and endodermal tissues, or organs of these origins. In the case of 18-hour embryos only ectodermal and mesodermal tissues could be counted, whereas in 60- and 72-hour embryos dividing cells were counted in neural tube (ectodermal), mesenchyme and mesodermal segments (mesodermal), and the lining of the gut (endodermal). A cell was counted as a dividing one when it was in any stage between early metaphase and late telephase. Late telephases were counted as two cells, and late prophases were not counted. The number of mitoses in a field was first determined, then the total number of cells in that field was calculated by inserting a disc divided into 36 equal squares into one of the

* Aided in part by NCI grant and in part by St. Louis Nadah Club Fund.

TABLE I. Effects of Cortisone and Growth Hormone on Mitotic Activity and Cell Density in 60-Hour Chick Embryo.

Procedure	No. of embryos	Ectoderm		Mesoderm		Endoderm	
		% mitoses	Cell density*	% mitoses	Cell density*	% mitoses	Cell density*
Cortisone acetate	6	8.8 ± 1.99	1.19 ± .66	5.0 ± .83	.89 ± .74	4.6 ± 1.23	.56 ± .63
		$p < .005$	$.30 > p > .25$	$.025 > p > .01$	$p > .25$		$p > .25$
Growth hormone	6	5.1 ± 1.41	2.23 ± .61	7.5 ± .76	1.63 ± 1.30	4.1 ± .85	1.10 ± .09
		$.025 > p > .01$	$.025 > p > .01$	$.025 > p > .01$	$.025 > p > .01$		$.025 > p > .01$
Sodium chloride	6	4.4 ± 1.20	1.32 ± .33	6.4 ± 1.15	1.11 ± .07	4.2 ± 1.53	.66 ± 1.03

* Cell density = $\frac{\text{Total No. cells}}{\text{No. of fields}} \times \frac{1}{100}$.

oculars of a binocular microscope, and estimating the total number of cells by counting the number of cells in several representative squares and multiplying by the appropriate factor to include the total number of squares occupying the field. Careful checks were made repeatedly to insure the correct number of dividing cells. During a large part of the study 2 persons performed counts independently, and the differences were slight. Mitotic activity in each instance was expressed as the percent of mitotic cells as shown in the 2 Tables. The average number of cells per field in each group was expressed as the cell density by a formula also shown in the Tables. The total number of cells examined averaged about 24,000 per tissue in each group of embryos examined up to and including 60 hours of incubation, and about 36,000 per tissue in each group of embryos after 72 hours incubation.

Results. Neither the mitotic counts nor the cell density determinations obtained in embryos up to 60 hours of incubation differed significantly as between control and hormone-injected groups. These data therefore have been omitted. At 60 hours of incubation cortisone had a significant effect in increasing mitotic activity in ectoderm, and in decreasing it in mesoderm, but had no effect on endoderm. There was no significant effect of cortisone on cell density in any of the 3 embryonal layers. Growth hormone significantly increased mitotic activity in ectoderm and mesoderm, but not in endoderm. It also had a significant effect in increasing cell density in all 3 embryonal layers. These data are shown in Table I.

As regards data obtained at 72 hours of incubation, comparisons have been made with the sodium chloride controls. Cortisone significantly diminished mitotic activity in ectoderm and mesoderm, but not in endoderm, and had no effect on cell density in any of the 3 embryonal derivatives. It is evident that at this stage growth hormone produced no mitotic stimulating effect but on the contrary showed some mitotic suppression in mesoderm. There was, however, a significant increase in cell density in ectodermal and mesodermal derivatives, but not in endoderm. Albumin showed some mitotic suppression of ectoderm and endoderm, but not of mesoderm, while showing no significant change in cell density in any of the 3 types of embryonal tissues. The 72-hour data are shown in Table II.

TABLE II. Effects of Cortisone and Growth Hormone on Mitotic Activity and Cell Density in 72-Hour Chick Embryo.

Procedure	No. of embryos	Ectoderm		Mesoderm		Endoderm	
		% mitoses	Cell density*	% mitoses	Cell density*	% mitoses	Cell density*
Cortisone acetate	7	7.1 ± .74	1.45 ± .58	5.1 ± .81	1.1 ± .73	6.1 ± 1.12	.67
		$p > .005$	$.15 > p > .10$	$p < .005$	$p < .3$	$.15 > p > .10$	
Growth hormone	8	10.9 ± 1.88	2.01 ± .09	6.2 ± .63	1.5 ± .32	5.4 ± .24	.71
		$.10 > p > .05$	$.0025 > p > .01$	$.025 > p > .01$	$.025 > p > .01$	$.05 > p > .025$	
Sodium chloride	6	12.3 ± 1.78	1.60 ± .47	7.8 ± 1.05	1.1 ± .15	7.3 ± 2.39	.75
Albumin	10	10.2 ± 1.51	1.38 ± .03	7.5 ± 1.07	1.1 ± .76	4.1 ± .80	.72
		$.025 > p > .01$	$.30 > p > .20$			$.025 > p > .01$	

* See footnote, Table I.

Discussion. The data on mitotic activity indicate cellular proliferative activity at or shortly before the time of fixation, while those on cell density may be taken as an indicator of mitotic activity in the past. The basic action of cortisone was to suppress mitotic activity, although there was a transient increase in ectoderm observed at 60 hours. On the other hand, the basic action of growth hormone was to increase cellular proliferation as shown by the data on mitotic activity as well as cell density. Such conclusions are valid insofar as they relate to saline controls; albumin proved to be toxic to young embryos, as evidenced by an extremely high mortality rate.

The observations with cortisone support the previous findings(1,5,6,7) and further establish the fact that embryonal cells of the ectoderm and mesoderm first become responsive some time between 60 and 72 hours of incubation. The observations on the effects of growth hormone in early embryogenesis are new and show that this hormone can stimulate mitotic activity in the ectoderm and mesoderm as early as 60 hours of incubation. However, this response is lost by 72 hours, at which time the effects of the previous increase in mitotic activity are expressed as an increase in cell density. Endoderm appears to be completely refractory to both of these hormones.

Since it is known that in all likelihood the development of a functional endocrine system does not occur in the chick embryo before the seventh incubation day, it would appear valid to conclude that these effects were not produced through indirect mechanisms such as the stimulation of synergistic or suppression of antagonistic hormones. Rather, it seems most likely that these are direct hormonal effects. Such a conclusion is also supported, in the case of cortisone, by its mitotic suppressing action on chick femur in tissue culture(8).

Summary. The effects of cortisone and pituitary growth hormone on embryonal tissues have been studied in chick embryos at time periods varying between 18 and 72 hours of incubation. No effects were noted at 18 and 30 hours. Mitotic counts have demonstrated the basic mitotic suppressing effect of cortisone in mesoderm at 60 hours and in ectoderm and mesoderm at 72 hours, although there were no changes in endoderm. An increase in mitotic activity was observed in embryos receiving growth hormone in the ectoderm and mesoderm at 60 hours, but not at 72 hours. However, an increase in cell

density was observed in these embryonal tissues at 72 hours of incubation. Endoderm was also refractory to this hormone.

1. Karnofsky, A. A., Ridgeway, L. P., Peterson, P. A., *Endocrinol.*, 1951, v48, 596.
2. Hsieh, K. M., Wang, T. Y., Blumenthal, H. T., *ibid.*, 1952, v51, 298.
3. Wang, T. Y., Hsieh, K. M., Blumenthal, H. T., *ibid.*, 1953, v53, 520.
4. Blumenthal, H. T., Hsieh, K. M., Wang, T. Y., *Am. J. Path.*, 1954 v30, 771.
5. Pikman, D. S., Ridley, A., Orgel, M., Blumenthal, H. T., *Endocrinol.*, 1959, v64, 790.
6. Evans, H. J., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 31.
7. Siegel, B. V., Smith, M. J., Gerstl, B., *A.M.A. Arch. Path.*, 1957, v63, 562.
8. Buno, W., Goyena, H., PROC. SOC. EXP. BIOL AND MED., 1955, v89, 622.

Received May 4, 1961. P.S.E.B.M., 1962, v109.

A Transmissible Spleen Weight Increase Factor (SWIF) of Mice.* (27173)

P. G. STANSLY, D. S. RAMSEY, AND C. F. NEILSON
(Introduced by C. S. Stulberg)
Detroit Institute of Cancer Research and Department of Microbiology, College of Medicine, Wayne State University, Detroit, Mich.

During investigation of the cell-free transmission of a reticulum cell sarcoma in BALB/c mice(1), an agent was encountered in certain spleen extracts whose main obvious effect was to cause a marked increase in size and weight of spleens of mice within a week. The mice otherwise appeared normal and continued in apparent good health indefinitely. This communication concerns some of the properties of the spleen weight increase factor (SWIF), now in its seventh passage, responsible for this activity.

Materials and methods. Cell-free extracts of the spleens of a transplantable reticulum cell sarcoma of BALB/c mice were the original source of the SWIF. Extracts were prepared by homogenizing tissue for 15 minutes at the highest speed of the Virtis "23" (23,000 rpm) apparatus surrounded by ice. The homogenate was centrifuged at 800 × *g* for 10 minutes, the supernatant removed by a bulb pipette and stored in a dry ice chest until use. Before inoculation it was frozen and thawed twice more, using dry ice and acetone. BALB/c, as well as a variety of other inbred lines, served as hosts for passage and to test for spleen weight increase activity. Mice of various ages and of both sexes, but primarily males (unless otherwise noted), were used. Within any experiment, however, strain, age, and sex were constant.

Generally, the average of 6 mice determined a single experimental point. The inoculum consisted of 0.1 ml of a 10% cell-free homogenate in phosphate buffered saline introduced intraperitoneally. The mice were sacrificed at 7 days by chloroform. The spleens were excised and weighed on a Roller-Smith balance, and average weight and standard error determined.

Results. A study of the time course of spleen weight increase in mice inoculated with the SWIF indicated that a maximum effect occurred at 7 days, and that the abnormality persisted at least 56 days, beyond which observations were not made.

Table I contains data showing the magnitude of the spleen weight increase. Lymph nodes were also increased in weight, though not to the same extent as the spleen. Other organs, such as the thymus, liver, lungs, and kidneys were not significantly affected. As shown in Experiment 2, Table I, the relative increase was the same whether the tissue was weighed wet or dry, suggesting that the increase in weight was due to an increase of cells rather than fluid. Indeed, in a preliminary experiment, a count of cells from sec-

* Aided by grants from Am. Cancer Soc., the United Foundation of Greater Detroit, administered through Mich. Cancer Foundation.