Effect of Adrenal Cortex Extract on Blood Cells of the Embryonic Chick. (17435)

EDWIN M. WELLER AND A. M. SCHECHTMAN

From the Department of Zoology, University of California, Los Angeles, Calif.

In a previous paper,[1] it was shown that a transient lymphopenia and concomitant leukocytosis occurs in the adult fowl after a single injection of adrenal cortical extract, thus resembling mammalian blood cell changes reported by others.[2]

The present study was undertaken to determine the response of embryonic blood cells to adrenal cortex extract (ACE). In addition, other effects of the extract were observed in the hope that we might add to the meagre information known on the role of the adrenal cortex in the embryo.[3]

Method. Single Comb White Leghorn chick embryos were used. Sterile 0.85% saline (control) and aqueous adrenal cortex extract* were injected into the allantoic cavity of embryos incubated 13, 14, 15 days, the dosage varying from 0.2 to 0.6 cc. Each embryo received only a single injection. One embryo series was bled from the vitelline vein[4] 24 hours after injection, while the second series was injected but allowed to hatch. In the first group, each embryo was bled once, and after bleeding was weighed to the nearest tenth gram, with extra-embryonic material removed.

Blood was diluted to the 101 mark in a certified RBC diluting pipette, the diluent[5] consisting of 25 mg of neutral red solution added to 100 cc of 0.85% saline. Total absolute counts per cubic millimeter (mm^3) of erythrocytes and leukocytes combined, were made in a Neubauer Brightline Counting Chamber.

Blood smears were stained with Wright's solution and 200 fields were examined on each smear. A Whipple disc was employed to determine the average number of cells in a field. Using a simple proportion, the actual count of a particular cell on a smear was converted to an absolute count:

$$\frac{\text{Smear count}}{\text{Avg No. cells in field} \times 200} = \times \frac{\text{(Absol. No. cells/mm}^3}{\text{Total absol. count/mm}^3}$$

In the second series, the hatched chicks were bled on alternate days from the ulnar vein, over a period of one month after hatching, and the weight of every specimen taken after each bleeding. Smears were stained in the usual manner and differential white blood cell counts were made by counting 200 leukocytes in equally distributed sections of each slide.

Results. The injection of 0.3 cc of ACE had no apparent effect on the number of lymphoid cells in the peripheral blood of chick embryos of 14 to 16 days incubation. However, the hormone had a potent influence on the polymorph leukocyte number (Table I). Applying the chi-square test[6] to the frequency distribution of lymphoid cells (lymphocytes and hemocytoblasts[7]) and polymorph leukocytes, showed that chance accounted for the lymphocyte distribution, whereas the presence of the cortical extract significantly affected the distribution of the leukocytes.

The blood cell count means are shown in Table II. A statistical analysis of the difference between the means of the saline and ACE groups was not undertaken because of

1 Shapiro, A. B., and Schechtman, A. M., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 440.

2 White, A., and Dougherty, T. F., *Ann. N. Y. Acad. Sci.*, 1946, **46**, 859.

3 Landauer, W., *Endocrinology*, 1947, **41**, 489.

* The extract was furnished through the courtesy of Dr. David Klein of the Wilson Laboratories.

4 Zifferblatt, A. N., and Seelaus, H. K., *Anat. Rec.*, 1931, **48**, 367.

5 Forkner, C. E., *J. Exp. Med.*, 1929, **50**, 121.

6 Waugh, A. E., Elements of Statistical Method, McGraw-Hill Book Co., 1943.

7 Fennell, R. A., *J. Agric. Res.*, 1947, **74**, 217.

TABLE I.

Effect of 0.3 cc ACE on Lymphocytes and Polymorph Leukocytes of Embryonic Chicks Injected on the 13th, 14th, and 15th Day of Incubation and Bled 24 Hours Later. Application of the Chi-Square Test.

Class mark (No. cells/mm³)	Observed frequency (f)		Expected frequency (f')		(χ^2) $(f-f')^2/f'$	
	Sal.	ACE	Sal.	ACE	Sal.	ACE
			Lymphocytes			
0 to 251	24	30	25.1	28.9	.048	.042
252 to 751	23	26	22.8	26.2	.002	.002
752 to 1251	9	10	8.9	10.1	.001	.001
1252 to 2251	12	10	10.2	11.8	.320	.271
2252 to 9751	7	10	7.9	9.1	.102	.089
Totals	75	86	74.9	86.1		.878*
			Polymorphonuclear leukocytes			
0 to 101	48	39	40.4	46.6	1.420	1.230
102 to 301	9	10	8.8	10.2	.004	.004
302 to 2101	8	26	15.8	18.2	3.850	3.340
Totals	65	75	65.0	75.0		9.848†

* $\chi^2 = .878$, 4 degrees freedom: $P = .95$ or chance accounted for 95% of the observed frequency distribution.

† $\chi^2 = 9.848$, 2 degrees freedom: $P = .01$ or ACE accounted for 99% of the observed frequency distribution.

TABLE II.

Effect of Single Injection of ACE and Saline (0.3 cc each) on the Blood Count of Embryos, Bled on the 14th, 15th, and 16th Day of Incubation (Injected 24 Hours Previously). Mean Values.

Cell (No./mm³)	14 days		15 days		16 days	
	Sal.	ACE	Sal.	ACE	Sal.	ACE
Lymphocyte	1,978	1,791	1,197	924	619	729
S.D.*	2,800	1,902	708	694	606	647
No. embryo	(27)	(34)	(16)	(18)	(32)	(34)
Polymorphs	106	309	108	319	152	416
S.D.	138	421	282	302	174	484
No. embryo	(17)	(23)	(16)	(18)	(32)	(34)
RBC† × 10^3	1,926	1,816	1,844	1,889	1,961	1,845
S.D.	268	297	187	229	334	328
No. embryo	(30)	(36)	(16)	(18)	(32)	(35)

* S.D. $= \frac{1}{N}\sqrt{N\Sigma(x^2) - \Sigma(x^2)}$, where N is the number of embryos, and x is the individual value of the mean.

† is equivalent to number of total cells in counting chamber.

the large standard deviation (S.D.) and the skewness of the distribution of the individual counts. The erythrocytes do not seem to have been affected by ACE since the mean total absolute count, consisting mainly of erythrocytes, was approximately the same in both treated groups during the incubation period considered.

Table III shows that the hormone did not affect embryo weight significantly, although a slight trend toward a weight loss was present in the 14 day hormone-injected embryos.

The presence of ACE depressed the hatchability rate of embryos in the second series (injected and allowed to hatch). Out of 154 saline-treated embryos, 82.4% hatched, while 68.4% of 149 hormone-injected embryos hatched. The critical ratio of the per cent

TABLE III.

Effect of Single Injections of ACE and Saline (0.3 cc each) on the Wet Weight of Embryos Weighed 24 Hr After Injection on the 14th, 15th, and 16th Day of Incubation. Mean Values.

	14 days		15 days		16 days	
	Sal.	ACE	Sal.	ACE	Sal.	ACE
No. embryos	(47)	(60)	(16)	(18)	(38)	(38)
Wet weight (g)	8.1	6.3	12.3	11.1	13.1	13.1
S.D.	1.0	1.2	0.9	1.0	1.1	1.5
S.E.*	1.53		1.33		1.85	
t	1.22		.90		.56	

* S.E. (standard error) $= \sqrt{\sigma M_1^2 - \sigma M_2^2}$, where σ is the standard deviation of M_1 and M_2, the means. $t = \frac{M_1 - M^2}{S.E.}$, $t = 2.2$ is significant.

difference was 2.86 which is statistically significant, since a ratio of 2.57 is equivalent to 99% confidence.

No discernible differences in the blood smears or weights of the hatched chicks between the 2 treated groups were observed. An apparent greater degree of barren skin area under the wings and abdomen and whiter down feathers were noticed in the ACE hatched chicks as compared to the control group.

Discussion. In the present study no change could be detected in the number of lymphoid cells after administration of adrenal cortical extract. However, there was a significant leukocytosis upon hormonal injection.

Failure to detect a transient lymphopenia is not in agreement with previous work on the adult fowl and mammals,[1,2] but we do not believe that such a conclusion is warranted in the present study. The relatively small number of lymphocytes on a smear and the resemblance of their precursors (hemocytoblast) to certain primitive erythrocytic cells,[7] renders the lymphocyte count less reliable than the counts of the more readily identifiable polymorphs. Furthermore, the large individual variation in lymphocyte numbers in the embryos would tend to obscure the hormonal effect.

Our failure to observe a lymphopenia after ACE injection might also be attributed to the length of time (24 hours) elapsing between injection of the hormone and removal of blood samples. Lymphopenia in adult mammals and fowl occurs at about 3-6 hours post injection. No lymphopenia was observed in 15 embryos from which blood was removed at intervals of 3, 6, or 9 hours after injection of ACE. The period of 24 hours was selected for most of the specimens in order to allow for a greater degree of absorption from the chorioallantois, although the possibility remains that a shorter period might have been more effective.

Erythrocytes showed no response to ACE which corresponds with results in the adult fowl.[1] Variation of the dose from 0.2 to 0.6 cc had no apparent effect on the general embryonic blood picture.

The weight loss of embryos injected with adrenal cortical extract, although not statistically significant, taken together with the definite decrease in hatchability, suggests that the hormone retarded embryonic metabolism. This may have been due to the extract's toxicity or perhaps to its protein catabolic action.[8] Landauer,[3] reported that the injection of either 0.02 or 0.05 cc of ACE into 0 or 120 hour chick embryos produced a very marked retardation of growth and, with the larger amount of hormone, none of the embryos completed development. The use of 11-desoxycorticosterone or other steroids having no effect on protein metabolism,[8] may reveal whether ACE is toxic, as it seems to be, to the embryonic organism.

Thiourea injected into chick embryos has been reported[9] to inhibit the thyroid, lower

8 White, A., Proteins and Amino Acids in Nutrition, Reinhold Publ., 1948, ch. 7.

9 McMenamin, J. W., unpublished M.A. Thesis, Univ. Calif., Los Angeles, 1946.

body weight and produce a feather syndrome similar to the one here reported. This suggests that ACE may act indirectly on the embryo by depressing the thyroid to the extent of interfering with local feather and general embryonic metabolism.

Summary. 1. The blood picture of 14, 15 and 16 day White Leghorn chick embryos injected into the allantois with aqueous adrenal cortex extract, shows no significant change in the number of lymphocytes but a marked absolute polymorph leukocytosis, 24 hours after injection. The erythrocytes are not affected by the extract.

2. Large individual variation in the lymphocyte count and greater difficulty involved in their identification may have obscured the effect of the hormonal preparation on the lymphocyte count.

3. ACE treatment induced a tendency (not statistically significant) toward weight loss in the 14 day embryos, a significant decrease in the hatchability rate, and an apparent defective feather development. However the blood picture and weight in comparison with the controls was normal after hatching.

Received September 7, 1949. P.S.E.B.M., 1949, **72**.

Glycerophosphatases of the Normal and Tumorous Frog Kidney. (17436)

THOMAS J. KING AND ROSS F. NIGRELLI

From the Department of Biology, Washington Square College, New York University, and the New York Aquarium, New York Zoological Society.

Lucké[1] described a neoplastic growth in the kidneys of the leopard frog, *Rana pipiens* Schreber (Fig. 1), which was manifested sometimes as a circumscribed adenoma and, at other times, as an adenocarcinoma with considerable infiltrative and destructive activities. The incidence, as reported by Lucké[1] and corroborated by the present studies, may be as much as 2% among both sexes.

It has been demonstrated that the alkaline phosphatase of the kidneys of many animals shows considerable activity.[2,3] Since it is further known that the phosphatase content of an organ is frequently altered under various pathological conditions[4,5] and especially in neoplasia[6] it was felt that studies of the alkaline and acid phosphatase systems might add to our knowledge of the physiology of the normal and pathological frog kidney.

Experimental procedure. Kidneys, both normal and tumorous were treated with a modified Gomori method recommended by Kabat and Furth.[7] Pieces of fresh kidney were fixed in cold acetone at 0°C for 24 hours and embedded in paraffin-Bayberry wax. Sections cut at 5 μ were mounted, deparaffinized and transferred to the buffered sodium glycerophosphate mixture and incubated for 8 hours at 37°C. Control sections were maintained. Some sections were stained in the usual way with Harris' hematoxylin and eosin; others were stained with safranin.

Tissues which were to be compared, namely, normal, neoplastic and calcium controlled sections were incubated and stained simultaneously.

Acid phosphatase was demonstrated by the Wolf, Kabat, and Newman[8] modification of Gomori's original method.[9]

Stewart[10] has pointed out that in cross sec-

1 Lucké, B., *Am. J. Cancer*, 1934, **20**, 352.

2 Kay, H. D., *Biochem. J.*, 1928, **22**, 855.

3 —— ——, *Physiol. Rev.*, 1932, **12**, 384.

4 Brain, R. T., and Kay, H. D., *Biochem. J.*, 1927, **21**, 1104.

5 Folley, S. J., and Kay, H. D., *Ergebn. d. Enzymforschung*, 1936, **5**, 159.

6 Greenstein, J. P., *J. Nat. Cancer Inst.*, 1943, **4**, 275.

7 Kabat, E. A., and Furth, J., *Am. J. Path.*, 1941, **17**, 303.

8 Wolf, A. E., Kabat, E., and Newman, W., *Am. J. Path.*, 1943, **19**, 423.

9 Gomori, G., *Arch. Path.*, 1941, **32**, 189.

10 Stewart, S. G., *Anat. Rec.*, 1927, **36**, 259.