In a table compiled by Marshall(17) the cardiac indices for various animals were: Dog, 2.86; goat, 3.07; rabbit, 1.69; and horse, 5.84. The mean cardiac index of 2.476 for the present group of normal ferrets is one intermediate between those for dog and rabbit.

Summary. The mean cardiac output per minute per square meter of body surface for a group of 10 normal ferrets was found to be 2.476 liters. The average circulation time by the fluorescein technic was 6.8 seconds and 4.5 seconds as determined by the cyanide method. The average mean arterial blood pressure was 147 mm Hg. Various other related data were also tabulated.

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Amount of Alkaline Phosphatase in the Oviduct of Folic Acid Deficient Chicks.* (17553)

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Increases in alkaline phosphatase in the reproductive tract following injection of estrogens have been described for many species. Hertz and Sebrell(1) first noted an impairment in the response to stilbestrol of the oviduct of chicks deficient in folic acid. This failure of estrogens to induce growth in the absence of folic acid has also been observed in the monkey,(2) frog,(3) and rat.(4) The following experiments were undertaken to determine whether the amount of alkaline phosphatase in the oviduct of the chick is affected by a folic acid deficiency.

Material and methods. Exp. 1. Eleven white leghorn chicks were obtained at hatching and placed in cages having elevated wire mesh floors. At 2 days of age 5 chicks were placed on a diet of chick mash, while the remainder were fed chick mash containing 2% folic acid antagonist.‡ Both groups received water *ad libitum*. Three birds in each group were kept as uninjected controls. The remaining chicks were injected subcutaneously with 50μg of estradiol§ in 0.05 ml of sesame oil daily for 5 days. The injections were started on the 12th day of feeding and the chicks were killed 24 hours after the last injection. At post mortem the oviducts were removed, weighed and placed in cold 80% ethyl alcohol. Histochemical studies for alkaline phosphatase were carried out by the method of Gomori,(5) in which the sections were incubated at 37°C for 2 hours with glycerophosphate and examined without the use of a counterstain. Body weights were recorded daily and blood samples obtained at autopsy for hemoglobin determinations.

Exp. 2. A total of 38 white leghorn chicks were obtained at time of hatching and treatment started at 2 days of age. Thirteen of the chicks received the normal diet, 13 received the normal diet containing 2% folic acid antagonist and 12 received a synthetic

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1. Hertz, R., and Sebrell, W. H., *Science*, 1944, v100, 293.

2. Hertz, R., PROC. SOC. EXP. BIOL. AND MED., 1948, v67, 113.

3. Goldsmith, E. D., Schreiber, S. S., and Nigrelli, R. F., PROC. SOC. EXP. BIOL. AND MED., 1948, v69, 299.

4. Hertz, R., and Tullner, Wm. W., *Endocrinol.*, 1949, v44, 278.

‡ We are indebted to Dr. E. L. R. Stokstad, Lederle Laboratories, New York, for the crude x-methyl folic acid used in these studies.

§ Estradiol was obtained through the courtesy of the Schering Corporation, Bloomfield, N.J.

5. Gomori, G., *J. Cell. and Comp. Physiol.*, 1941, v17, 71.

TABLE I.
Effect of Methyl Folic Acid on Deposition of Alkaline Phosphatase after Treatment with Estradiol.

No. of chicks	Diet	Estradiol, μg	Body wt, g	Oviduct wt, mg	Alkaline phosphatase
3	Control	—	95	17.3	0
2	"	50	112	29.0	3+
3	Antagonist	—	70	13.2	0
3	"	50	68	16.2	1+

TABLE II.
Effect of Crude Methyl Folic Acid and a Folic Acid Free Diet on Deposition of Alkaline Phosphatase after Treatment with Stilbestrol.

No. of chicks	Diet	Stilbestrol, mg	Body wt, g	Oviduct wt, mg	Alkaline phosphatase
6	Control	—	157	17.3	3.7+
7	"	0.5	159	436.3	3.7+
5	Synthetic	—	79	12.8	1.6+
7	"	0.5	71	78.5	1.7+
6	Antagonist	—	73	12.2	1.9+
7	"	0.5	68	69.2	0.7+

diet(6) supplemented with all the known vitamins except folic acid. Starting on the 13th day of feeding the special diets, 7 chicks in each of the 3 diet groups received 0.5 mg stilbestrol daily for 5 days. All chicks were killed 24 hours after the last injection and autopsied as in Exp. 1. In order to obtain information with regard to the relative amount of phosphatase present under the various conditions of the experiments an arbitrary scale was established ranging from 0 reaction for tissue totally devoid of phosphatase to a 4+ reaction for tissue showing a maximum response. In both experiments the same scale was used and the evaluation made only on the phosphatase noted in the endometrium.

Results. The chicks on the control diet showed a normal increase in body weight throughout the experiments whereas the birds on the synthetic diet and the diet containing the folic acid antagonist showed negligible increase in weight after the 9th day. Thus the final body weight of the chicks in Exp. 1 on antagonist diet was 69 g while the controls showed a body weight of 101 g. Similarly the control chicks in Experiment 2 reached a final weight of 158 g while the weight of the folic acid deficient chicks varied from 68 to 79 g (Tables I and II). Hemoglobin values varied widely; however, an average drop of 10% was obtained in the folic acid deficient birds. In Exp. 1, treatment with estradiol caused a 68% increase in the oviduct weight of the normal bird but had no effect on the weight of the oviduct of the chicks receiving the folic acid antagonist. Phosphatase occurred to a marked degree in the control chicks which showed a 3+ reaction and only to a slight degree, *i.e.*, a 1+ reaction, in the birds receiving the antagonist (Table I).

In Exp. 2 the higher dosage of estrogen used gave a much greater effect on the weight of the oviduct. The oviducts of the control chicks treated with stilbestrol weighed 436.3 mg whereas the oviducts of the chicks on the synthetic and antagonist diets weighed 78.5 and 69.2 mg respectively (Table II). The alkaline phosphatase was also markedly reduced in the oviducts of these last two groups. It is apparent from an examination of Fig. 1 that a large amount of alkaline phosphatase is present in the chicks on the normal diet whereas the folic acid deficient chicks show a marked reduction in the enzyme regardless of stilbestrol treatment.

Discussion. The present study confirms the work of Hertz(7) and others that folic acid is necessary for the growth-promoting

6. Campbell, C. J., Brown, R. A., and Emmett, A. D., *J. Biol. Chem.*, 1944, v152, 483.

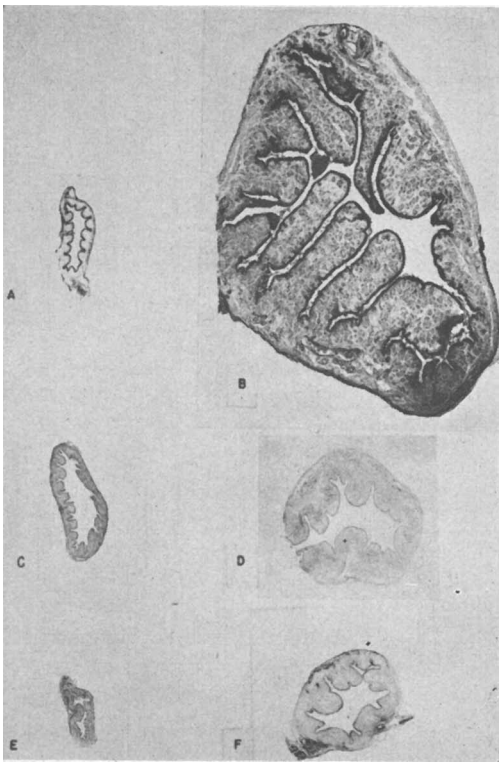

FIG. 1.

Sections through oviducts, all × 32. Note that the heavy deposition of alkaline phosphatase present in the endometrium of A and B, fails to occur in folic acid deficiency (C-F).

A—Uninj. control.
B—Normal diet. Inj. with stilbestrol.
C—Synthetic diet.
D—Synthetic diet. Inj. with stilbestrol.
E—Antagonist diet.
F—Antagonist diet. Inj. with stilbestrol.

action of estrogen on the reproductive tract. The mechanism whereby this vitamin acts in the growth of the tissue is as yet unexplained.

It has been suggested that folic acid may function as a coenzyme in the synthesis of thymine-like compounds which are then utilized in the production of nucleic acid.(8) Partial support for this concept is seen in the work of Prusoff *et al.*(9) who showed that a decrease in desoxyribonucleic acid occurred when *Lactobacillus casei* is grown on a folic acid deficient medium. It has also been shown that aminopterin not only inhibited the estrogen induced growth of the oviduct of the frog but caused an increase in the number of mitotic figures, conceivably by a reduction in nucleic acid and consequent retardation in cell division.(10) The present results indicate that folic acid may also be essential for the deposition and maintenance of alkaline phosphatase in the reproductive tract. If this enzyme is necessary for the growth-promoting action of estrogen then its absence would explain the failure of estrogens to induce growth. Thus it is possible that the absence of folic acid as induced under the conditions of this experiment interferes with the increase in phosphatase following estrogen treatment and prevents normal growth response.

Summary. A folic acid deficiency produced by feeding a synthetic diet or the addition of crude x-methyl folic acid to a normal diet markedly decreases the amount of alkaline phosphatase in the oviduct of chicks after estrogen treatment.

7. Hertz, R., *Recent Progress in Hormone Research*, II, 1948, Academic Press.

8. Stokes, J. L., *J. Bact.*, 1944, v48, 201.

9. Prusoff, W. H., Teply, L. J., and King, C. G., *J. Biol. Chem.*, 1948, v176, 1309.

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