

TABLE III.
Incidence of Cecitis in Rats Fed *Salmonella* Enteritidis, and in Untreated Controls.

Degree of cecitis in rats aged 3 mo.		Degree of cecitis in rats aged 4 mo.		Degree of cecitis in rats aged 5 mo.	
Test animals	Controls	Test animals	Controls	Test animals	Controls
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	0	0
0	0	0	0	?	0
?	0	0	0	++	0
?	0	?	0	+++	0
?	0	?	0	+++	0
?	0	++	0	+++	0
+	0	++	?	++++	0
+	?	++	++++	++++	?

0 = No ulcer, no glands, no cysts.

? = No ulcer, slight or doubtful enlargement of glands, no cysts.

+ = Ulcer to 0.5 cm, enlarged glands and/or cysts.

++ = Ulcer 0.5 to 1.0 cm, enlarged glands and/or cysts.

+++ = Ulcer 1.0 to 2.0 cm, enlarged glands, cysts, adhesions, and matting.

++++ = Huge ulcers, enlarged glands, cysts, adhesions, and matting.

organisms. Of special interest is the fact that in spite of the extensive infestation of the colony healthy carriers are of greatest rarity.

Summary. 1. A specific strain of salmonella has been invariably obtained on culture from the ulcers of rat cecitis. 2. *Salmonella* were not obtained from the cecum in the absence of lesions with one exception. 3. Agglutinins for the specific strain of salmonella

are found in most of the rats in this colony although the titer is on the average higher in animals with visible cecitis. 4. Feeding the specific salmonella in appropriate doses was followed after a long latent period by development of cecitis in many of the test animals. 5. The association of some other synergistic agent is not fully excluded but seems at present unlikely.

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17224. Aminopterin and Response of Frog Oviducts to Estradiol. II. Histological Studies and Mitotic Counts.*

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In a previous paper,¹ the gross changes in the immature female frog oviduct following parenteral administration of estradiol benzoate[†] alone and in conjunction with folic acid[‡] and with a folic acid antagonist, amin-

opterin,[‡] were described. Aminopterin (4-amino pteroylglutamic acid) inhibited the growth and differentiation response of oviducts of newly metamorphosed female frogs, *Rana clamitans*, to estradiol. The aminopter-

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¹ Goldsmith, E. D., Schreiber, S. S., and Nigrelli, R. F., *Proc. Soc. Exp. Biol. and Med.*, 1948, **69**, 299.

[†] The estradiol benzoate was generously supplied by Dr. F. F. Yonkman and Dr. F. L. Mohr of Ciba Pharmaceutical Products.

[‡] The 4-amino pteroylglutamic acid was generously supplied by the late Dr. Y. SubbaRow, and the folic acid by Dr. T. H. Jukes and the late Dr. A. L. Franklin of Lederle Laboratories.

in effect could not be overcome by folic acid in concentrations as high as 100 parts of folic acid to 1 part of aminopterin. In the light of more recent work, it is probable that pre- and concurrent treatment with higher dosages of folic acid would have reversed the antagonist action.

Animals were fixed in Bouin's solution and serial sections of oviducts extending along the cranial half or two-thirds of the kidney were cut at 7 μ and were stained with hematoxylin and eosin, iron hematoxylin, or with Masson's stain. Mitotic counts were made of the oviduct epithelium; sections from each slide were examined so that comparable areas in all the animals were studied in order to obtain estimates of mitotic activity throughout the length of the oviduct.

Results. In the untreated controls, the small and uncoiled oviducts consisted of a single layer of cuboidal epithelium with regular oval or round nuclei which encircled a small lumen. Mitotic counts of the oviduct epithelium revealed less than one mitotic figure per thousand cells. After the administration of estradiol for 2 to 3 weeks (0.1 mg per week), there was marked increase in the diameter, length and coiling of the oviduct. The bulk of the oviducts consisted of glands which radiated from the oviduct lumina to the periphery. The gland cells contained basal nuclei and at times granular cytoplasm. The lumina were lined with well differentiated ciliated columnar epithelium with regular oval, vesicular nuclei. Mitotic figures were rare, and in over 12,000 cells the incidence of mitotic figures was less than one per 1500 cells. Addition of folic acid to estradiol accentuated the growth and differentiation response of the oviducts, but the mitotic counts were not significantly different from those animals receiving only estradiol.

Either pretreatment with aminopterin or simultaneous treatment of the frogs with aminopterin and estradiol appeared to inhibit the estrogen response. The oviducts remained uncoiled, increased in diameter only slightly and showed areas of cystic dilatation. There was no evidence of gland formation, and a single layer of low columnar epithelium lined the lumina which were only slightly larger

than those in untreated controls. In the areas of cystic dilatation, the epithelium around the lumina was flat and almost squamous in appearance. There was a significant increase in mitotic figures in these oviducts, with 12 to 36 figures per thousand cells. Although metaphases predominated, there were considerable numbers of the other mitotic phases.

Except for some slightly increased infolding of the oviduct epithelium into the lumina, supplementation of the aminopterin and estradiol with folic acid did not significantly change the histological picture characteristic of those receiving only the antagonist and estradiol. Mitotic counts in all but one animal revealed 17 to 46 mitotic figures per thousand cells. In 2 animals, one receiving aminopterin and estradiol, and the second receiving folic acid as well, where there was no significant increase in mitoses over the controls, the nuclei of the oviduct epithelium were markedly abnormal, with great variations in size and shape. The rest of the histological picture was no different from other animals treated similarly.

Discussion. The results indicate that although the growth and differentiation of the oviducts were inhibited by aminopterin the number of mitotic figures was significantly increased. It must be emphasized that the administration of estradiol alone is followed by an increase in the number of cells and mitoses. However, at the time of sacrifice, 2 to 3 weeks after initiation of treatment, the most rapid growth had passed its peak. At the end of this period, the oviducts under the influence of the antagonist and the estrogen had grown but slightly, but a large number of mitotic figures were present.

This apparently paradoxical situation of a picture of numerous mitotic figures and very little growth may be resolved by the following considerations.

From the observations of Brachet² and Painter,³ Koller⁴ has adduced that the amount of available nucleic acid is one of the principal factors which determine the rate of cell di-

² Brachet, J., *Arch. Biol.*, Paris, 1940, **51**, 151.

³ Painter, T. S., *Proc. Nat. Acad. Sci.*, 1940, **26**, 95.

⁴ Koller, P. C., *Nucleic Acid*, Cambridge Univ. Press, 1947, p. 280.

vision. It was suggested⁵ that folic acid may function as a coenzyme in a system responsible for the synthesis of thymine or a thymine-like compound which is then utilized in the synthesis of nucleic acid. Further evidence of this relationship is to be seen in *Lactobacillus casei* which when grown in a folic acid deficient medium showed a marked decrease in deoxyribonucleic acid,⁶ a universal constituent of cell nuclei.⁷

It is tempting to speculate that in the presence of aminopterin, a folic acid antagonist, the folic acid utilization in the organism may be interfered with, resulting in a reduction in nucleic acid synthesis and a marked retardation of the rate of cell division. These facts might serve to explain the growth inhibition obtained with the folic acid antagonists in

certain of the leukemias^{8,9} and in the transplantable mouse Sarcoma 180.¹⁰

Summary. Macroscopically and microscopically, oviducts of frogs receiving aminopterin (4-amino pteroylglutamic acid) failed to respond to the growth stimulating action of estradiol. However, a significantly greater number of mitotic figures were visible in these oviducts. Evidence is presented to suggest that the folic acid antagonists may exert their growth inhibitory action by interfering with folic acid utilization in the organism, with a consequent reduction in nucleic acid synthesis and a retardation of the rate of cell division.

⁸ Farber, S., Diamond, L. K., Mercer, R. D., Sylvester, R. F. Jr., and Wolff, J. A., *New England J. Med.*, 1948, **238**, 787.

⁹ Burchenal, J. H., Burchenal, J. R., Kushida, M. N., Johnston, S. F., and Williams, B. S., *Cancer*, 1949, **2**, 113.

¹⁰ Moore, H. E., Stock, C. C., Sugiura, K., and Rhoads, C. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1949, **70**, 396.

⁵ Stokes, J. L., *J. Bact.*, 1944, **48**, 201.

⁶ Prusoff, W. H., Teply, L. J., and King, C. G., *J. Biol. Chem.*, 1948, **176**, 1309.

⁷ Stedman, E., and Stedman, E., *Nucleic Acid*, Cambridge Univ. Press, 1947, p. 232.

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17225. Increased Metabolic Rate in Rats after X-Irradiation.

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Some metabolic reactions may be increased after exposure to total-body irradiation. Indirect evidence for increased metabolism in dogs 1-3 weeks after irradiation was presented by Prosser *et al.*:¹ (1) Weight loss was greater after irradiation than in non-irradiated dogs in which food intake was limited to amounts less than consumed by the irradiated animals. (2) Nitrogen excretion was maintained by irradiated dogs despite a decrease in food intake, and in the acute terminal period the nitrogen balance was negative. (3) The metabolic balance minus the

water balance or metabolic index is given by $[(\text{food} + \text{water} + \text{weight loss}) - (\text{urine} + \text{feces})] - [(\text{food water} + \text{water drunk}) - (\text{urine} + \text{feces water})]$. This metabolic index is a measure of CO₂ exhaled plus water of metabolism and water of tissue breakdown. This quantity increased significantly in dogs during the first 2 weeks after irradiation and increased considerably during the acute terminal period.

As a check on the hypothesis that catabolism is increased after irradiation, basal metabolism has been measured in rats after total body exposure to x-rays.

Materials and methods. Forty-four albino rats, 24 males and 20 females, ranging in initial weight from 160 to 310 g, (average 206 g) were used. There was no apparent dif-

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¹ Prosser, C. L., with contributions by Painter, E. E., Lisco, H., Brues, A. M., Jacobson, L. O., and Swift, M. N., *Radiology*, 1947, **49**, 299.