*therapeutic effect* of repeated doses. This effect is obvious from the experiments reported in the literature indicating that *fractions* of the curative dose, repeated at such intervals as to leave the organism without penicillin for the greatest part of the treatment period, result in cure. This is the case in spirochetal[11] as well as in bacterial[3] infections. The *additive effect* might depend on biological changes induced by sub-curative doses on the micro-organism, on reduction of the bacterial population, and on the immunological response of the host. The experiments of Kelly and Schnitzer[12] would suggest furthermore that the immunological response might influence the susceptibility of the micro-organism to chemotherapy.

The immunological response itself can be influenced by the treatment schedule, according to the recent observations of Kilbourne and Loge.[13] In hemolytic streptococcal pharyngitis, the antistreptolysin titer is higher with *intermittent* penicillin injections than it is with continuously sustained blood levels.

Another phenomenon which accounts for the independence of therapeutic effect duration from blood level is revealed in the experiments of Grunberg, Schnitzer, and Unger.[14] Penicillinase injected *after* the total disappearance of penicillin from the host tissue still blocks the therapeutic effect in the local streptococcal infection of mice. Thus, the presence of the penicillin in the micro-organism is more prolonged than in the host.

In our own experiments, part of the therapeutic effect existing beyond the blood level is due to penicillin accumulated in the tissues. How much of this prolonged therapeutic effect is dependent upon the undetectable amounts present in the blood, in comparison with the penicillin accumulated in the organs and tissue fluids, remains an open question.

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[12] Kelly, D. R., and Schnitzer, R. J., *Arch. Biochem.*, 1945, **7**, 461.

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## 4-Amino Pteroylglutamic Acid (Aminopterin), Pteroylglutamic (Folic) Acid, and Response of Frogs, *Rana clamitans*, to Estrogens.*

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It has been shown that chicks and monkeys (*Macaccus rhesus*) maintained on a diet deficient in pteroylglutamic (folic) acid exhibit a decreased response to estrogenic substances.[1-3] It has been further demonstrated by Franklin, Stokstad, and Jukes[4] that folic acid deficiency in mice may be accelerated by a chemical antagonist of folic acid ("crude antagonist", Lederle). Hertz,[5] using the same antagonist, has shown that it interferes with the estrogen response of the chick genital

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[1] Hertz, R., *Endocrinology*, 1945, **37**, 1.

[2] Hertz, R., Recent Progress in Hormone Research, II, Academic Press, 1948, 161.

[3] Hertz, R., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 113.

[4] Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 368.

[5] Hertz, R., *Science*, 1948, **107**, 300.

TABLE I.

Effects of Various Levels of 4-Aminopteroylglutamic Acid, Pteroylglutamic Acid, and Estradiol Benzoate on the Growth of Oviducts of *Rana clamitans*.

| Series No. | No. of frogs | Aminopterin per inj., mg | Folic acid per inj., mg | No. of inj. per wk | Estradiol per wk, mg | Oviduct response of female frogs: After 2 wks of estradiol | Oviduct response of female frogs: After 3 wks of estradiol |
|---|---|---|---|---|---|---|---|
| I* | 9 | .05 | 0 | 3 | .1 | + | + |
| " | 10 | .10 | 0 | 3 | .1 | + | + |
| " | 5 | 0 | 2.0 | 3 | .1 | +++ | ++++ |
| " | 5 | .10 | 1.0 | 3 | .1 | + | + |
| " | 10† | 0 | 0 | 0 | .1 | +++ | ++++ |
| " | 5 | 0 | 0 | 0 | 0 | — | — |
| II* | 5 | .05 | 0 | 3 | .1 | + | |
| " | 13 | .10 | 0 | 3 | .1 | + | |
| " | 11 | .20 | 0 | 3 | .1 | + | |
| " | 2 | 1.0 | 0 | 2 | .1 | + | |
| " | 5 | 0 | 1.0 | 3 | .1 | ++++ | |
| " | 5 | 0 | 2.0 | 3 | .1 | ++++ | |
| " | 5 | .05 | 1.0‡ | 3 | .1 | + | |
| " | 4 | .1 | 2.5‡ | 3 | .1 | + | |
| " | 5 | .25 | 2.5‡ | 3 | .1 | + | |
| " | 5 | 0 | 0 | 0 | 0 | — | |
| " | 5 | 0 | 0 | 0 | .1 | +++ | |

* Series I animals were simultaneously treated with estradiol and with substance(s) listed. Series II animals were pretreated for 2-3 weeks with the drug(s) listed and then in addition were injected with estradiol. (Administration of the drug(s) aminopterin, folic acid, or both were continued throughout estradiol treatment.)

† 5 animals of this group also received 0.4 cc of distilled water 3 times per week.

‡ Folic acid level was raised after 2 weeks treatment to 5 mg per injection.

\+ Slight oviduct enlargement, no coiling.

+++ Oviducts enlarged and coiled.

++++ Marked oviduct enlargement and coiling.

— Oviducts small, thin, and uncoiled.

tract. The 4-amino analogue of pteroylglutamic acid was described as being strongly antagonistic to folic acid in the *Streptococcus fecalis* R test.[6] Goldsmith, Tobias, and Harnly[7] reported that both the "crude antagonist" and 4-amino pteroylglutamic acid (aminopterin) prevented the development of *Drosophila melanogaster* larvae into adults. This inhibition by the "crude antagonist" could be overcome in a number of cases by the addition of folic acid to the diet. However, thus far, the inhibitory effect of aminopterin on the development of Drosophila larvae has not been counteracted or reversed by several levels of folic acid. Franklin, Stokstad, and Jukes[8] found that mice died within a few days after being placed on a diet containing 1 mg or more of aminopterin per kilo of diet. At a level of 0.3 mg per kilo the aminopterin depressed the blood values. This could be reversed by high dosages of folic acid. However, large amounts of folic acid did not overcome the effect resulting from higher concentrations of aminopterin. Recently, Hertz described the decreased response of the chick[9] and the rat[10] to estrogen following treatment with aminopterin. High levels of folic acid reversed this effect.[10]

In the light of the above data, and since it had been established previously that estradiol injections in newly metamorphosed frogs are followed by a pronounced oviduct response within 2 to 3 weeks,[11] it was considered worth-

6 Seeger, D. R., Smith, J. M., Jr., and Hultquist, M. E., *J. Am. Chem. Soc.*, 1947, **69**, 2567.

7 Goldsmith, E. D., Tobias, E. B., and Harnly, M. H., *Anat. Rec.*, 1948, **101**, 104.

8 Franklin, A. L., Stokstad, E. L. R., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 398.

9 Hertz, R., Proc. 39th Annual Meeting Am. Assn. Cancer Research, March 1948.

10 Hertz, R., personal communication.

11 Schreiber, S., and Rugh, R., *J. Exp. Zool.*, 1945, **99**, 93.

while to ascertain the action of aminopterin and its effect on the estrogen response in frogs.

*Experimental.* This paper presents preliminary data on a group of about 100 newly metamorphosed frogs (*Rana clamitans*) treated parenterally with various dosage levels of aminopterin, folic acid, and estradiol benzoate. The course of treatment and levels of drugs administered are outlined in Table I. The frogs were divided into two series. In series I, the frogs were treated with aminopterin† or folic acid,† or both, and were simultaneously treated with estradiol benzoate.‡ In series II, frogs were pretreated with aminopterin alone, folic acid alone, or both for a period of 2 to 3 weeks. At this time, estradiol was added to this regimen. The volume of fluid injected was controlled as closely as possible, and no frog received more than 0.4 cc of fluid per injection. In addition, the site of puncture was carefully held closed to prevent any leakage of injected fluid.

The data indicate that treatment with aminopterin decreased the response of the female frog oviducts to estradiol. Oviducts of the estradiol treated females showed marked enlargement and coiling. However, the oviducts of the females which received estradiol and aminopterin simultaneously, or which were pretreated with aminopterin and then were given estradiol plus aminopterin, showed very slight enlargement and no coiling.

Simultaneous administration of folic acid and estradiol was followed by oviduct growth and coiling in the females equal to, but not greater than that observed in the females treated with estradiol alone. However, pretreatment with folic acid for 2 to 3 weeks followed by injections of estradiol plus folic acid resulted in oviduct growth which was greater than that observed in the females which received only estradiol.

In 4 groups of frogs, in addition to aminopterin and estradiol, supplements of folic acid were administered in ratios of 10, 50 and 100 to 1 of folic acid to aminopterin. In none of these female frogs were the oviducts grossly different from those in the females treated only with aminopterin and estradiol.

The data also indicate that those animals treated with aminopterin appeared to show a higher mortality rate than those receiving supplements of folic acid in addition to aminopterin. The majority of aminopterin treated frogs which died, did so 3 to 4 weeks following initiation of treatment with aminopterin, irrespective of level of drug administered.

Histological studies of the oviducts and other organs such as liver, spleen, and adrenal will be reported at a later date.

*Summary.* 4-amino pteroylglutamic acid (aminopterin) markedly decreased, whereas, folic acid increased the response of the oviducts of newly metamorphosed female frogs (*Rana clamitans*) to estradiol. The aminopterin effect could not be reversed by folic acid in ratios as high as 100:1 of folic acid to aminopterin.

† The 4-amino pteroylglutamic acid was generously supplied by the late Dr. Y. SubbaRow, and the pteroylglutamic acid by Dr. A. L. Franklin of Lederle Laboratories.

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