# Further Role of Antibiotics in Aminopterin-Induced Folic Acid Deficiency in Rats.* (19433)

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In previous experiments(1) we have shown that the "folic acid deficiency" produced in rats by the use of the folic acid antagonist aminopterin can be overcome by the peroral or intraperitoneal administration of aureomycin. However, the "folic acid deficiency" produced in rats fed a sulfonamide drug was not corrected by aureomycin. Since preliminary experiments showed that the citrovorum factor concentrates were able to correct the deficiency produced on either type of diet, it seemed probable we were dealing with a citrovorum factor deficiency rather than a folic acid deficiency. It was suggested that aminopterin is probably more accurately a citrovorum factor antagonist than a folic acid antagonist.

Further experiments were done to provide information on the mechanism of action by which aureomycin corrected the citrovorum factor deficiency. A number of other antibiotics were studied since it was of interest to test further the hypothesis that the beneficial effect of these substances is mediated through their influence on the intestinal flora.

*Experimental.* Male rats of the Sprague-Dawley strain, 24 days old, were given a synthetic diet consisting of sucrose 73 parts; vitamin-free casein (Labco), 18 parts; salt mixture,† 4 parts; corn oil, 5 parts; thiamine, 0.5 mg; riboflavin, 0.5 mg; niacin, 1 mg; calcium pantothenate, 2 mg; pyridoxine, 0.5 mg; i-inositol, 100 mg; biotin, .002 mg; choline, 300 mg; para-amino benzoic acid, 25 mg; vitamin K, 0.5 mg. Two drops of oleum percomorphum were given by mouth twice weekly to each rat as a source of vit. A and D. Five rats were in each group and each series was repeated at least once. A total of 225 rats were used in these experiments.

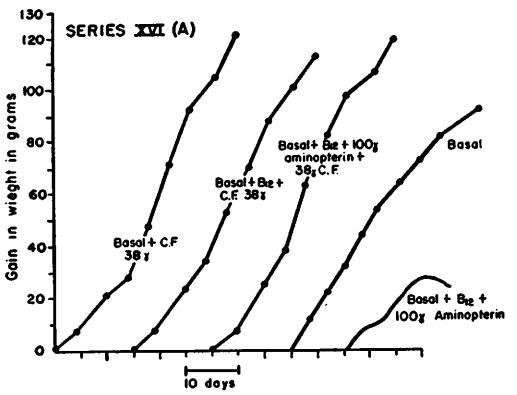


FIG. 1. Effectiveness of citrovorum factor in counteracting aminopterin.

*Results.* The data extend the previous findings that the addition of citrovorum factor can correct the "folic acid deficiency" induced by aminopterin, and that aureomycin is able to counteract the effect of the antagonist. Fig. 1 illustrates the weight gain obtained on a basal diet without any folic acid compounds, and the increased growth that results after citrovorum factor‡ is added. The further addition of vit. $B_{12}$ was without effect and it may be that adequate quantities of $B_{12}$ occur in the purified casein so that no increased growth response could be demonstrated with additional vit. $B_{12}$. It will also be seen in Fig. 1 that when 100 $\mu$g of aminopterin‡ are added to the diet containing 38 $\mu$g of citrovorum factor the antagonist is unable to produce the deficiency. From other work in this laboratory it is apparent that small amounts of citrovorum factor can counteract increasing amounts of aminopterin in a stoichiometric relationship. It should be remembered that this relationship does not exist between folic acid and the folic acid antagonists.

Fig. 2 shows the poor growth obtained with

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† Salts No. 2, Nutritional Biochemicals, Inc.

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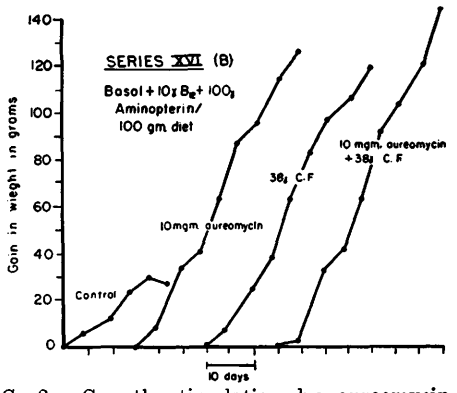


FIG. 2. Growth stimulation by aureomycin or citrovorum factor in aminopterin fed rats.

100 μg of aminopterin on the basal diet containing vitamin $B_{12}$ and the markedly improved growth which results when 10 mg of aureomycin are included in such a diet. If 38 μg of citrovorum factor are added to the basal aminopterin diet, growth is about equal to that of the aureomycin group. This suggests that aureomycin probably does influence the bacterial flora in such a way that citrovorum factor or folic acid is provided to the host. Fig. 2 also demonstrates that when aureomycin and citrovorum factor are added together, despite the presence of aminopterin, growth is much superior to that of either aureomycin or citrovorum factor alone. It may be that when citrovorum factor is added to the diet conditions are made more favorable for the intestinal bacteria to provide still further growth factors which have not yet been elucidated.

We have found that different sources of rats can influence some of the findings in our experiments. Evidently the strain of rats, and more importantly their intestinal flora, play a part in determining what growth response the animal will have. It has been found that the rats from commercial source "A" did not provide the consistent growth response obtained with aureomycin on the aminopterin diet. More reproducible results were experienced with rats from commercial source "B". Aureomycin was able to correct the aminopterin-induced deficiency in 2 of 3 series, but in the third irregular responses were obtained. This last group of rats was obtained from the commercial source "A" and our experience with these animals indicated a variable growth response in rats receiving the same diet. When this was recognized, only rats from commercial source "B" were used.

Since the beneficial effect of aureomycin was suggestive, it was of interest to study the effect of other antibiotics on aminopterin-induced folic acid deficient diets. Fig. 3 shows that streptomycin, penicillin, terramycin and chloromycetin are unable to counteract the effect of aminopterin. In 2 of the series terramycin was ineffective although in a third series the increased weight gain was indicative of the improvement over the aminopterin-containing diet. This last observation emphasizes the variations in the sources of rats and also illustrates the importance of the previous nutritional history of the breeding females.

*Discussion.* These experiments have provided additional information on the action of antibiotics in the deficiency induced by a folic acid antagonist. Although we feel that the antagonist probably induced a folic acid and/or citrovorum factor deficiency, no final explanation exists as to how the antibiotics are able to overcome these particular deficits. There is some evidence to support the hypothesis that the intestinal flora is providing the factors required by the host. Davis and Chow(2) gave indirect evidence when they showed that radioactive cobalt ($CO^{60}$) in the diet of adult rats could be incorporated in the vit. $B_{12}$ found in the feces after aureomycin was fed. Jacob and his coworkers(3) found that intravenous aureomycin was re-excreted into the intestines and partially suppressed the fecal flora in both man and dog.

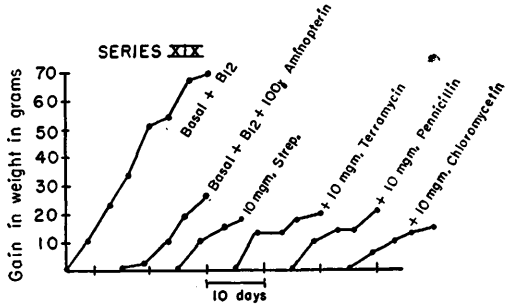


FIG. 3. Inability of 4 antibiotics to counteract effect of aminopterin in rats.

It is of interest, therefore, to speculate why the beneficial effect was not obtained with antibiotics other than aureomycin, since their antibacterial spectra are not too dissimilar. It may be that some of the antibiotics were rapidly destroyed in the intestinal tract or by body tissues and therefore could not be effective against the "deleterious" bacteria that were either consuming citrovorum factor or destroying it and therefore making it unavailable to the host. One of the greatest defects in our information at present is the fact that little is known about the synthetic abilities of a particular microorganism and less information is available on the interrelationships between the various organisms in the intestinal tract. The suppression of some bacteria and/or the resulting ability of others to flourish may account for the relative effectiveness of an antibiotic to indirectly provide the particular substance required by the host.

*Summary.* Streptomycin, terramycin, penicillin and chloromycetin, unlike aureomycin, were ineffective in overcoming the deficiency induced by the feeding of aminopterin to rats. Citrovorum factor is able to counteract aminopterin at low levels, indicating that the aminopterin may be a citrovorum factor antagonist as well as a folic acid antagonist. The addition of aureomycin to a diet containing citrovorum factor provides better growth than could be obtained on either supplement alone.

1. Waisman, H. A., Green, M., Cravioto, J., Ramenchik, A., and Richmond, J. B., PROC. SOC. EXP. BIOL. AND MED., 1951 v76, 384.

2. Davis, R. L., and Chow, B. F., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 218.

3. Jacob, S., Schweinburg, F. B., and Rutenburg, A. M., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 121.

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## Normal Mucoprotein Values in the Dog; Response to Distemper Vaccine and to Endocarditis.* (19434)

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The alterations in serum proteins in a variety of clinical conditions have received considerable attention in recent years. One field of investigation has concentrated on the mucoprotein constituents of the blood serum. It has been shown by several investigators (1-5) that an elevation in the mucoprotein concentration in the human occurs in many conditions. Winzler and coworkers(1,2) have demonstrated an increase in patients with malignancies of various types. They likewise showed that patients with pneumonia have an elevated mucoprotein level. Other infections, both of bacterial and of virus origin, are reported to be accompanied by a rise in mucoprotein values(3). It would appear that the mucoprotein component of the blood exhibits a non-specific response to body tissue injury. The only instance of reduction in the mucoprotein value clinically is that which occurs with severe renal disease(5). Recently, attention has been directed to the response of the mucoproteins in rheumatic fever(3,4,6). Adams and coworkers(6), on the basis of studies in rheumatic fever patients, have suggested that even though adrenocorticotropin therapy often produced an apparent clinical and laboratory remission, the abnormal mucoprotein elevations persisted in their patients

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