

Effect of Phthalylsulfathiazole on Growth and Reproduction of Rats Fed a Soybean Protein Ration.* (18097)

M. O. SCHULTZE

From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.

The possibility of an improved performance of animals consuming bacteriostatic or bactericidal agents was clearly visualized and expressed by Moore *et al.*(1). They observed increased weight gains of chicks when streptomycin or sulfasuxidine were added to a purified ration. More recently, Stokstad and Jukes(2) found that the feeding of aureomycin to chickens stimulated their growth. Similarly, marked increases in weight gains of pigs fed plant rations have been reported as a result of the addition of aureomycin(3,4) and of streptomycin(5) to these rations. While the reasons for these effects of the antibiotics are at present largely a matter of conjecture it is quite possible that their beneficial effects are intimately related to their action on the microflora of the intestinal tract.

Miller(6) and Kon(7) have reviewed much of the evidence which supports the view that insoluble sulfonamides, including phthalylsulfathiazole (PST) exert their effect on the animal organism primarily through modification of the intestinal flora. A temporary(8)

or more permanent(6,9) depression of the number of coliform organisms in the intestine can apparently be compensated by an increase in other species so that the total bacterial count may not be reduced(6,8). The depression of growth(10), the granulocytopenia and leucopenia(11), the interference with reproduction(12) and lactation of rats (13) caused by sulfonamides can be overcome by feeding sufficient quantities of folic acid with the diet. With an adequate intake of folic acid and biotin the growth(10,14,15) and reproductive performance of rats are not impaired(16). In fact, improved growth performance of rats maintained on adequate natural or purified diets containing insoluble sulfonamides has been observed(10,15,17). However, Jones *et al.*(18) reported that the growth depressing effects on rats fed rations containing 5% of sulfasuxidine could not be completely overcome by folic acid unless liver extract was also supplied. Recent observations made in this laboratory in connection with another study(19) provide evidence for the favorable effect of a bacteriostatic compound on the nutrition of a mammalian species. In the experiment re-

* This is the fourth of a series of papers dealing with the nutritional value of plant materials. Paper No. 2429, Scientific Journal Series, Minnesota Agricultural Experiment Station.

1. Moore, P. R., Evenson, A., Luckey, T. D., McCoy, E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1946, v165, 437.

2. Stokstad, E. L. R., and Jukes, T. H., *Proc. Soc. Exp. Biol. and Med.*, 1950, v73, 523.

3. Jukes, T. H., Stokstad, E. L. R., Taylor, R. R., Cunha, T. J., Edwards, H. M., and Meadows, G. B., *Arch. Biochem.*, 1950, v26, 327.

4. Carpenter, L. E., *Arch. Biochem.*, 1950, v27, 469.

5. Luecke, R. W., McMillan, W. N. and Thorp, F., Jr., *Arch. Biochem.*, 1950, v26, 326.

6. Miller, A. K., *J. Nutr.*, 1945, v29, 143.

7. Kon, S. K., *Proc. Nutr. Soc.*, 1945, v3, 217.

8. Gant, O. K., Ransone, B., McCoy, E., and Elvehjem, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1943, v52, 276.

9. Ellinger, P., *Experientia*, 1950, v6, 144.

10. Welch, A. D., and Wright, L. D., *J. Nutr.*, 1943, v25, 555.

11. Daft, F. S., and Sebrell, W. H., *U. S. Public Health Reports*, 1943, v58, 1542.

12. Nelson, M. M., and Evans, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1947, v66, 289.

13. Nelson, M. M. and Evans, H. M., *Arch. Biochem.*, 1948, v18, 153.

14. Shehata, O., and Johnson, B. C., *Proc. Soc. Exp. Biol. and Med.*, 1948, v67, 332.

15. Spector, H., *J. Biol. Chem.*, 1948, v173, 659.

16. Nelson, M. M., and Evans, H. M., *Proc. Soc. Exp. Biol. and Med.*, 1948, v68, 274.

17. Baxter, J. H., *J. Nutr.*, 1947, v34, 333.

18. Jones, J. H., Rogers, C. S. and Stone, C. H., III, *J. Nutr.*, 1949, v39, 579.

19. Schultze, M. O., *J. Nutr.*, 1950, v41, 103.

TABLE I. Effect of Phthalylsulfathiazole on Growth, Reproduction, and Acute Uremia of the Newborn.

	Basal ration	Basal ration + 2% PST
No. of female rats	15	15
6 weeks post weaning wt gain (g)	109.9 ± 4.9*	114.1 ± 3.5*
No. of sterile females	0	1
No. of pregnancies	38†	26†
No. of litters born alive	37	26
Mean No. of young born per litter	7.03 ± 0.40*	7.65 ± 0.59*
% of young born dead	5.0	1.0
Mortality of young 0-4th day %	37.2	6.1
Mortality of young 0-21st day %	53.4	32.9
Incidence of acute uremia of newborn; % of litters	35.1	0
Mean No. of young weaned per litter survivors	5.2 ± 0.49*	5.7 ± 0.51*
Mean 21 day wt per survivor (g)	34.1 ± 0.42*	36.5 ± 0.45*
Litters with 6 or more young at 21 days	13	11
Mean No. of young in these litters	6.8	8.2
Mean wt change of mothers during lactation (g)	-1.2 ± 3.0*	+14.6 ± 3.3*

* Standard error of the mean.

† The larger number of pregnancies on the basal ration is due to the fact that 14 litters died within 96 hours after birth and their mothers were immediately bred again.

ported in the present paper phthalylsulfathiazole (PST) was fed to rats maintained on a ration containing a commercial soybean protein and DL-methionine as the sole source of dietary amino acids. The effect of such a regime was evaluated by observations on postweaning weight gains, on the reproductive performance and on the incidence of "acute uremia of the newborn" (20) among the litters produced.

Experimental. The basal ration (S₂) used has been previously described (19). It consisted of a commercial soybean protein (Archer-Daniels-Midland Co.), DL-methionine, sucrose, hydrogenated vegetable oil, corn oil, salts and suitable supplements of all available vitamins except ascorbic acid and vitamin B₁₂. In the ration of half of the animals 2% of PST (Sharp and Dohme, veterinary sulfathalidine) replaced an equal quantity of sucrose. The rations were fed *ad libitum*. The rats used for these experiments were F₂ or F₃ generation offspring from mothers maintained continuously on the basal ration. Carefully matched littermate females were divided at 21 days of age into 2 groups and fed the rations referred to above. The rats were housed in groups of 3-4 animals in cages with raised wire screens. When the females were 10 weeks old they

were bred with males maintained on the same rations. Shortly before parturition and during lactation the females were transferred to individual cages containing clean wood shavings. After weaning or loss of a litter the rats were returned immediately to the breeding cages. The experiment was terminated when most of the females on the PST ration had weaned 2 litters.

Results and discussion. The data summarized in Table I suggest that the addition of 2% of phthalylsulfathiazole to the ration of these rats supported better performance by every criterion applied. However, the differences in favor of the ration containing the bacteriostatic compound are statistically significant ($P < 0.05$) only with respect to the incidence of acute uremia of the newborn, the 21 days weight of the young and the weight change of the mothers during lactation.

The most striking effect of the addition of PST to the basal ration used in this experiment was the elimination of the syndrome of acute uremia of the newborn. This condition was encountered in the group consuming the basal ration with about the same incidence as in other, more extensive experiments (21). While the etiology of acute uremia of the newborn is not clear the syndrome can be

20. Schultze, M. O., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 613.

21. Halvorson, H. O. and Schultze, M. O., *J. Nutr.*, 1950, in press.

largely prevented by the feeding of materials known to be high in vit. B₁₂ activity(21) to the mothers or by injection of vit. B₁₂ into the young(20). The results presented in Table I could therefore be interpreted as indicating that in the presence of PST the intestinal flora was altered so that microbiological synthesis of vit. B₁₂ provided the maternal organism with a supply of this compound sufficient to prevent the appearance of the external symptoms of acute uremia in the newborn rats. Since absolutely specific methods for the determination of vit. B₁₂ are not available at present a decision as to whether microbiological synthesis of vit. B₁₂ was in fact responsible for the beneficial effects of PST reported here must be held in abeyance. The components of a ration may have a marked effect on gastrointestinal synthesis of vitamins(22). Thus, it has been reported(23) that the feces of rats maintained on an all plant ration were persistently negative for vit. B₁₂ activity while those pro-

duced on a casein ration contained vit. B₁₂ activity. In this connection it might be pointed out that the ceca of the rats consuming PST were in this experiment always found to be greatly enlarged compared to those of animals consuming the basal ration. This was observed even in 21-day-old weanling rats which had consumed the ration for only a few days. Baxter(17) has recorded a similar observation.

Summary. The feeding of 2% of phthalylsulfathiazole to rats maintained on a purified ration containing a commercial soybean protein and DL-methionine as the only source of amino acids produced no depression of growth; it supported better reproductive performance and no cases of acute uremia of the newborn were observed among about 200 rats. On the basal ration, acute uremia of the newborn occurred in 35% of the litters born.

The assistance of Merck and Company, Abbott Laboratories, Lederle Laboratories and du Pont and Company with various supplies is gratefully acknowledged.

22. Wright, L. D., Skeggs, H. R. and Sprague, K. L., *J. Nutr.*, 1945, v29, 430.

23. Zucker, T. F. and Zucker, L. M., Abstracts 117th Meeting Am. Chem. Soc., 1950, p. 16A.

Received July 17, 1950. P.S.E.B.M., 1950, v75.

Effect of ACTH on Glycogenesis and Glycolysis in Hypophysectomized Rats. (18098)

L. G. ABOOD* AND J. J. KOCIS (Introduced by J. M. Coon)

From the Department of Pharmacology, University of Chicago, and Biochemical Section, Armour Research Laboratory, Armour and Co., Chicago.

Abelin(1) showed that after feeding starved rats a high carbohydrate diet, an increase in liver glycogen accompanied a 25% reduction in adrenal cholesterol. He suggested that the rise in blood sugar may have stimulated the pituitary to secrete ACTH, which caused a release of adrenocortical hormone, and this, in turn, stimulated liver glycogenesis. Ingle(2) was able to demonstrate

adrenal hypertrophy in rats which died as a result of an excessive carbohydrate diet. Similar results were reported by other workers (3,4). Hypophysectomy caused a decrease in the muscle glycogen of rats, but the glycogen level was restored to normal upon the administration of anterior pituitary extract; however, pure ACTH was without effect(5).

* Present address: Department of Physiology, University of Chicago.

1. Abelin, I., *Schweiz. med. Wochschr.*, 1946, v76, 527.

2. Ingle, D. J., *Endocrinology*, 1946, v39, 43.

3. Foglia, V. G., *Rev. soc., Argent. biol.*, 1945, v21, 45.

4. Bennett, L. L., and Koneff, A. A., *Anat. Rec.*, 1946, v96, 1.