snugly, with the dog standing up. Remove the wire plug from the tubing, insert a 21-gauge needle and again inject some heparin-saline solution. Insert a plug in the hilt of the needle and clamp the hilt on the supporting post (*8b* in Fig. 1).

Place dog in the cage, above which the assembled perfusion apparatus has already been suspended. Connect the metal conduit tubing to the supporting post (*8a*) by means of the bayonet lock. Remove the plug from the needle and insert the adapter tip of the tubing of the drip apparatus. Open the adjustable screw clamp to allow drops of fluid to flow at a chosen rate.

Discussion. The design of this apparatus is similar to one developed in this laboratory several years ago and used successfully in studies on effects of ammonium chloride acidosis on insulin action(5) and on glucose tolerance(6). In the earlier version a bicycle chain was used to support the tubing between reservoir and connection to the indwelling intravenous catheter, to allow up and down movements of the dog while protecting against twisting of the tubing, as was first employed by Jacobs(1). The flexible metal conduit as now used serves the same purpose but is simpler and permits easier assembly of the tubing than did the bicycle chain.

Summary. This report describes a simple apparatus for continuous intravenous infusion of fluids in dogs, employing a standard assembly of reservoir bottle and flexible connecting tubing that leads to the dog's harness and a plastic catheter dwelling in an external jugular vein. Suspension of the apparatus by a swivel above the dog's cage permits the dog almost complete freedom of movement during long periods of continuous infusions.

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Some Effects of Aureomycin and Penicillin on Thiamine and Riboflavin Metabolism in Growing Rats.* (24101)

N. B. GUERRANT AND J. M. STEEL
Department of Agricultural and Biological Chemistry, Pa. State University, University Park, Pa.

Investigators have found that certain antibiotics stimulate growth in young animals of several species but the mode of action has not been fully elucidated. It has been shown that the resulting growth response depends on the antibiotic used, animal species, composition of diet, and on sanitary condition of the environment. In general, antibiotics are less effective in stimulating growth when fed with nutritionally complete diets to animals maintained under sanitary conditions. These observations suggest a relationship between growth-promoting effect of antibiotic and intestinal flora of the animal. Lih and Baumann (1) reported vitamin-sparing action occurring in rats subsisting on antibiotic-containing diets sub-optimum in certain water-soluble vitamins. They reported penicillin to be more effective in stimulating additional growth in rats maintained on thiamine or riboflavin-deficient diets than are other antibiotics whereas in pantothenic acid deficiencies penicillin was least effective. Sauberlich(2) reported the thiamine-sparing action of aureomycin or penicillin to be greater in rats receiving dextrin-containing diets than in rats receiving sucrose-containing diets. He also reported the effect of these antibiotics to be greater when

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used in connection with thiamine deficiencies than with riboflavin or pyridoxine deficiencies. Johnson *et al.*(3) suggested the function of penicillin in sparing dietary thiamine to be through increased synthesis of the vitamin. Guggenheim *et al.*(4) investigated the effect of parenterally and orally administered antibiotics. According to these authors, antibiotics administered subcutaneously exhibited no significant growth-stimulating in animals receiving sub-optimal levels of thiamine, riboflavin, or pantothenic acid. When given orally to rats receiving sub-optimal levels of thiamine, penicillin and terramycin stimulated additional growth whereas aureomycin and streptomycin failed to do so. However, when given orally to riboflavin and pantothenic acid-deficient rats, penicillin, aureomycin, streptomycin, and terramycin were effective. Oral administration of antibiotics also affected excretion of vitamins. Linkswiler *et al.*(5) reported that orally administered aureomycin stimulated growth in rats receiving diets deficient in the B_6 vitamins, whereas Waisman *et al.*(6) found that this antibiotic overcame citrovorum factor deficiencies induced by feeding aminopterin. Much experimental work with antibiotics concerns growth of test animal when antibiotics are administered under controlled conditions. Except the efforts of Guggenheim *et al.*(4), little seems to have been done toward establishing a relationship between vitamin intake of test animal and that stored in tissues or that eliminated in excreta in presence or absence of ingested antibiotics. The present report contains some of the results obtained with rats relative to thiamine and riboflavin absorption and retention in the presence and absence of dietary aureomycin and penicillin.

Methods. Rats 20 to 28 days of age and weighing from 36 to 53 g were used. All rats except normal controls were partially depleted of body-store of thiamine, riboflavin, or thiamine and riboflavin, before being arranged on the basis of sex, weight, age, and genetic background into comparable experimental groups. The basal diet consisted of: vitamin-free casein 17%, hydrogenated cotton-seed oil 5%, salt mixture (U.S.P.) 4%, fiber (CellU-flour) 2%, cod liver oil 1%, vitamin pre-mix 1%, and sucrose 70%. The vitamin pre-mix contained 100 g : alpha tocopherol 2.5 mg, pyridoxine hydrochloride 0.25 mg, p-amino benzoic acid 20 mg, niacin 2 mg, choline chloride 100 mg, inositol 20 mg, calcium pantothenate 2 mg, folic acid 0.4 mg, Vit. K 0.2 mg, Vit. B_{12} 0.004 mg, and biotin 0.025 mg, dispersed in finely-pulverized casein. The antibiotics were incorporated into the basal diet at the rate of 100 ppm. When 2 antibiotics were incorporated into the same diet, 100 ppm of each were used. The 2 antibiotics employed were: penicillin (Procaine penicillin—Merck and Co.) and aureomycin (Lederle Laboratories). The supplementary vitamins (thiamine and riboflavin) were fed orally as freshly-prepared acidulated aqueous solutions. Experimental diets were prepared at biweekly intervals and stored under refrigeration until fed *ad libitum*. Rats were maintained in individual all-metal cages having raised-screen floors except during periods of excreta collecting. During the latter periods, they were maintained in individual metabolism cages permitting quantitative collection of urine and feces. Rats were weighed daily and food-consumption data were recorded weekly. Excreta from individual animals were collected daily, weighed or measured and preserved by means of 0.1 N hydrochloric acid and refrigeration. Four to 8 animals were used in each dietary treatment. At end of feeding periods, animals were sacrificed and liver and ceca (including contents and 10 mm of intestines on portal and ventral side) were dissected out, pressed between filter papers, weighed, macerated in presence of 0.1 N hydrochloric acid, and transferred to volumetric flasks. Feces were similarly treated. Urine, feces and body tissues were subjected to hydrolysis, made to volume with dilute acid, and aliquots of solutions were adsorbed on appropriate columns, eluted, and eluates assayed for thiamine and riboflavin by fluorometric procedures using Coleman Model 12 fluorometer.

Results. Data are reported (Table I) relative to food intake, body-weight increase, and amounts of thiamine and riboflavin recovered from liver, ceca, urine, and feces of rats following ingestion of known amounts of the 2

TABLE I. Some Effects of Penicillin and Aureomycin on Thiamine (B_1) and Riboflavin (B_2) Metabolism in the Growing Rat.

Animal group No.	I		II		III		IV		V		VI		VII		VIII	
Sub-group	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Previously depleted of	$B_1 + B_2$				B_1				B_2				B_1		B_2	
Daily thiamine intake (μg)*	2	2	1	1	2	2	12.5	12.5	12.5	12.5	12.5	12.5	2	2	12.5	12.5
" riboflavin " " *	0	0	4	4	25	25	25	25	1	1	25	25	25	25	2	2
Type of antibiotic fed†	P	None	P	None	P	None	P	None	P	None	P + A	None	A	None	A	None
Total food consumed (g)‡	89	69	81	49	95	81	151	194	187	166	215	208	120	121	147	127
" wt incr. (g)‡	31	11	28	7	31	18	72	100	45	45	88	82	18	17	22	12
Food consumed/wt incr.‡	2.9	6.3	2.9	7	3.1	6.2	2.1	1.9	4.2	3.8	2.4	2.5	6.7	7.1	6.7	10.6
Thiamine conc. (μg/g sample)																
Liver	2.4	1.6	1.3	0.7	1.7	1.1	3.4	2.5	3.2	3.6	3.1	3.4	1.1	1.2	3.9	3.6
Ceca	.2	.7	.2	.8	1.4	.7	.5	.9	.6	.6	.4	.8	.4	.4	.9	1.8
Feces	1.4	4.8	2.5	4.2	1.6	3.4	1.8	2.2	2.5	3.6	1.3	3.5	.8	1	2.1	3.8
Urine	.2	.2	.3	.4	2.9	1.2	1.7	.6	.2	.8	1.3	.8	.2	.1	.4	.3
Total thiamine (μg)‡																
Intake	40	40	20	20	40	40	250	250	250	250	250	250	40	40	250	250
Feces + urine	5.9	13.9	7.5	10.6	7.5	13.3	10.6	17.8	17.2	24.2	12.7	23.5	4	4	12.3	14.2
Liver + ceca	7.4	4.9	4.8	2	7.7	6.6	19.9	17.7	20.2	22.5	21.7	20.6	6.1	7.3	20.2	20.9
Riboflavin conc. (μg/g)																
Liver	8	7.6	8.8	10.8	13.8	26.6	16.9	16.3	11.8	11.2	15.4	14.6	20.8	18.5	9.8	8.8
Ceca	2	3	1.9	6.2	4.4	5.5	4.8	5.6	2.3	2.5	2.5	2.8	2	1.7	2.6	3.6
Feces	6.6	11.6	5.4	12.4					6.4	7.1	10	11.6	15.3	22.8	5.9	7.5
Urine	7.8	5.	4.3	5.6					2.6	3.2	10.3	7.3	26	20.9	6.7	8.4
Total riboflavin (μg)‡																
Intake	0	0	80	80	500	500	500	500	20	20	500	500	500	500	40	40
Feces + urine	46	51	29	44					59	61	113	114	121	113	56	50
Liver + ceca	28	25	36	26					69	74	112	139	93	118	51	47

* Supplements fed daily 20 days. † A = Aureomycin; P = Penicillin. ‡ Avg value for 21-day test period.

vitamins in presence and absence of the 2 antibiotics. The data reported are average values for the several rats constituting the respective experimental groups.

Discussion. In 6 out of 8 experiments, rats receiving diets supplemented with antibiotics consumed more food and grew at a more rapid rate (increase in body-weight) than did those receiving unsupplemented diets. Efficiency of food utilization likewise was highest among rats receiving antibiotic-supplemented diets in 6 experiments out of 8. In the other 2 experiments, where thiamine intake was high, food efficiency was slightly higher on the control diets. In all experiments antibiotics decreased the total amount of ingested thiamine recovered from the liver, ceca, feces, and urine. However, in no instance was the total amount of thiamine recovered equal to that ingested. Antibiotic supplementation increased thiamine content of the livers in 5 of 8 experiments and decreased the thiamine content of the ceca in 5 experiments. Antibiotics decreased the thiamine content of feces in all experiments and increased the thiamine content of the urine in 5 of 8 experiments. In 5 of 6 experiments, antibiotics decreased the total amount of ingested riboflavin accounted for in liver, ceca, urine, and feces of rats. When low dosages of riboflavin were given (0, 1, and 2 μg daily), the total amount of vitamin accounted for in the 4 sources was greater than the amount ingested. It is not clear whether this indicates synthesis of vitamin or whether the difference may have been due to residual riboflavin unremoved by previous depletion. If riboflavin synthesis took place, the synthesis was not influenced by presence or absence of the antibiotics. In 6 of 8 experiments, concentration of hepatic riboflavin was highest in rats having received the antibiotic-supplements, the difference being greatest where high intakes of the vitamin were involved. Concentration of riboflavin in ceca and the feces was generally lowest in rats that received the antibiotic supplements. However, in 3 experiments urinary riboflavin was highest for these rats.

Summary. 1. The data demonstrate that both penicillin and aureomycin supplementation increased food intakes of rats subsisting on diets deficient in either or both thiamine and riboflavin, but neither antibiotic influenced food consumption when adequate thiamine and adequate riboflavin was administered. In general, growth of test animals paralleled food consumption, with indications that antibiotic supplementation improved food utilization, particularly when thiamine intake was sub-optimum. 2. The data further indicate that the amount of thiamine accounted for was increased by feeding rats diets containing either penicillin or aureomycin, or a combination of the 2 antibiotics. However, the data show that thiamine absorption was improved in the presence of antibiotics, particularly, penicillin, as indicated by lower thiamine concentration in ceca and feces, and by higher thiamine concentration in liver and urine. 3. While the data do not exclude the possibility of riboflavin synthesis in the animal body, they do indicate that if synthesis occurred it was not influenced by the presence or absence of the antibiotics under investigation. There was evidence of increased riboflavin absorption in the presence of antibiotics as indicated by lower concentrations of vitamin in ceca and feces and a higher concentration in liver.

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