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Effects of Aureomycin on Renal Lesions, Liver Lipid, and Tissue Choline in Choline Deficiency. (19641)

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The impression was obtained in a previous study(1) that it might be possible, by causing certain alterations in the conditions in the gut, to reduce considerably the damage produced in rats by diets deficient in choline. Succinylsulfathiazole was studied at that time, but it was not observed to be effective in modifying the damage, although it did produce enlargement of the caeca. More recently, with the same idea in mind, the newer antibiotics have been examined. The present report presents evidence that the severe renal injury which occurred regularly as a result of choline deficiency was prevented in most cases by aureomycin. Fatty changes in the liver also appeared to be diminished somewhat and alterations in tissue and fecal choline were observed. However, it was not demonstrated conclusively that these effects of aureomycin were actually results of changing the conditions in the gut and not results of a direct metabolic effect.

Methods and materials. The animals employed were male rats of a Sherman strain, which were 20 to 23 days old and weighed 30 to 40 g when started on the experimental diets. The basal diet was the same as that described previously(1) with the carbohydrate in the form of sucrose, and with Wesson's modification of the Osborne-Mendel salt mixture with Zn and Co added in most experiments. The unsupplemented diet contained negligible amounts of choline, and presumably contained relatively small amounts of vit. B_{12}. The animals were maintained in individual cages with large-mesh screen bottoms, in a room with controlled temperature. The diets were fed *ad libitum* in some experiments. In others, each animal received 3.6 or 5.0 g of food each afternoon. In the latter experiments, the food was consumed completely and the food intake of all animals was the same. The supplements under study were incorporated in the diet in most cases in the form of pure, crystalline substances. After 10 days on the diets the surviving animals were autopsied after decapitation, and the results evaluated. It had been observed previously that few additional animals developed renal lesions or died after this period. In 4 experiments with 8 animals in each group, employing controlled feeding, total liver lipid, after 4 days on the diets, was determined gravimetrically as the petroleum ether-soluble portion of the material obtained by wet alcohol-ether extraction of homogenized tissue. Choline was determined by the method of Glick(2), either directly on the material extracted by alcohol-

TABLE I. Effects of Various Antibiotics and Vitamins on Development of Renal Lesions and Mortality (Experiments with *Ad libitum* Feeding).

Supplement to choline-free diet	No. of animals	Avg wt gain,* g/d	Animals with renal lesions		Animals with normal kidneys, %
			Dead, %	Alive, %	
0	67	2.5	77	22	1
Chloromycetin .1%	13	2.5	85	15	0
Penicillin 2500 u†	13	2.7	69	31	0
Aureomycin .1%	13	2.8	31	69	0
" .5%	26	1.8	4	8	88
Saline .2 cc†	28	2.9	64	36	0
B_{12} 2 μg in .2 cc saline†	21	2.9	52	43	5
B_{12} 60 μg/kg	12	2.4	50	50	0
Vit. E × 10	13	2.5	92	0	8
Vitamins, H_2O-sol., × 4‡	12	2.5	83	17	0
Choline .3%	10	3	0	0	100

* Wt gain during pre-lesion period (first 4 days on diet).
† Given as daily intraper. inj. Other supplements incorporated in diet.
‡ All water-soluble vitamins of diet, not including choline or B_{12}.

ether or after resolution by petroleum ether. Feces from controls and aureomycin-treated animals were collected into alcohol during the second, third and fourth days on the diets, and total choline determinations were carried out on the material obtained by Soxhlet extraction with methanol or by wet extraction after homogenization.

Results. Effects on renal lesions. The results of experiments with *ad libitum* feeding are summarized in Table I. Almost all of the animals on the unsupplemented diet developed the characteristic large tense "hemorrhagic" kidneys of choline deficiency, beginning near the end of the fourth day, and most of them died during the experimental period. Chloromycetin at 0.1% level, and penicillin in large doses, did not appreciably alter the results. On the other hand, aureomycin at 0.1% level appeared to decrease mortality, and at 0.5% level it largely prevented renal lesions and death of the animals. The data in the lower portion of Table I show that renal injury and death on the choline-deficient diet were not substantially affected by increasing greatly the amounts of vit. B_{12}, vit. E, or the remaining water-soluble vitamins of the diet. These results presumably indicate that the protection afforded by aureomycin was not entirely a result of conserving or increasing the production of any of these vitamins. The addition of choline to the diet resulted in complete protection. It should be noted that daily food consumption and gain in weight were reduced in the animals receiving the higher level of aureomycin, and it is well known that a sufficient reduction in food consumption will prevent development of renal lesions on a choline-deficient diet. However, we have observed in previous experiments that the daily weight gain must be reduced below 1 g or prevented entirely in order to achieve the degree of protection afforded by the aureomycin. Experiments with controlled feedings were carried out to clarify this question further.

In Table II, the results of experiments in which each animal received 3.6 g of food per day are summarized. The average daily gain in weight of the animals in each group was very nearly the same. The limitation in food consumption caused some delay in development of the renal lesions in the control group, as well as a moderate reduction in the incidence and severity of the lesions, compared with the results of experiments with *ad libitum* feeding. Again, however, aureomycin afforded an impressive degree of protection. Chloromycetin, terramycin, and streptomycin all prevented death, perhaps by controlling secondary infections, but only streptomycin appeared to reduce the incidence of the renal lesions and this reduction was much less than that observed with aureomycin. All of these antibiotics caused considerable enlargement of the caecum; this effect was slightly less marked with aureomycin than with the others. Chloro-

TABLE II. Effects of Various Antibiotics and Vitamins on Development of Renal Lesions and Mortality (Experiments with Controlled Feeding).*

Supplement to choline-free diet†	No. of animals	Animals with renal lesions		Animals with normal kidneys, %
		Dead, %	Alive, %	
0	77	38	44	18
Chloromycetin .6%	13	0	100	0
Terramycin .5%	10	0	80	20
Streptomycin .5%	11	0	55	45
Aureomycin .5%	59	0	20‡	80
" (reppt.)§ .6%	13	0	8	92
" (inact.)‖ .6%	13	54	46	0
B_{12} 60 μg/kg	13	15	31	54
Vit. E × 10	13	8	84	8
Choline .3%	5	0	0	100

* Each animal received 3.6 g of food/day.

† Concentrations of supplements calculated on basis of free base in cases where salts were employed.

‡ One-half of animals classified here as having renal lesions had very mild lesions.

§ Reprecipitated 3 times from acid solution.

‖ Inactivated by heating in alkaline solution.

mycetin produced diarrhea in some animals.

Effects of purification and alteration of aureomycin. When crystalline aureomycin HCl was further purified by repeated reprecipitation from acid solution, there was no reduction in its protective effect. On the other hand, when it was modified by heating in alkaline solution to the point of destroying its antibacterial action(3), its protection against choline deficiency was abolished, and it no longer caused enlargement of the caecum. These observations suggested the possibility of a relationship between the protective effect of aureomycin and its effects on the microorganisms in the gut. Under the circumstances of limited food consumption, vit. B_{12} appeared capable of affording definite protection, but less than that afforded by aureomycin.

Effects on other organs. The data in Table III, obtained from animals receiving 3.6 g of food per day, indicated that no change in size occurred in the thymus, adrenals, or thyroid glands as a result of administration of aureomycin. Similarly, no changes were observed on examination of microscopic sections of these organs. These studies made it appear unlikely that the protective action of aureomycin was due to effects on the thyroid or adrenals; the possibility of such a mechanism was suggested by our previous observation that thiouracil or cortisone afforded considerable protection against development of the renal lesions on a choline-deficient diet(4). In addition to producing the caecal enlargement already noted, aureomycin usually caused a reduction in the relative size of the liver (Table III).

Effects on liver lipid. The accumulation of lipid in the liver which occurred as a result of the choline-deficient diet appeared to be decreased slightly to moderately by aureomycin. The average value for total liver lipid in the untreated deficient groups was 9.5%, compared with 7.6% in the aureomycin-treated groups, and 5.0% in the groups receiving choline.

Effects on tissue choline. In Table IV are shown the results of an experiment on the effects of aureomycin on the choline concentration in liver and kidney tissue of animals on the deficient diet. It appeared from this and several other experiments that the tissue choline concentrations probably were increased to some extent by aureomycin.

Effects on fecal choline. Feces from aureomycin-treated animals in 4 experiments contained small but appreciable amounts of choline—0.3 to 1.5 mg per 2- or 3-day pool from 15 rats—while feces from animals on the unsupplemented choline-deficient diet contained no measurable quantities. Feces from animals receiving choline in the diet, like those from animals receiving aureomycin, were

TABLE III. Relative Wt of Organs.

	Diet (3.6 g/rat/day)			
	Choline-free† 4 days	Choline-free +.5% aureomycin 4 days	Choline-free +.5% aureomycin 9 days	Choline-free +.3% choline 9 days
Wt of organs (% of body wt)				
Liver	5.36	4.39	4.10	4.48
Kidneys	1.08	1.05	1.00	1.02
Heart	.50	.48	.40	.43
Thymus	.29	.25	.28	.29
Adrenals	.024	.026	.023	.023
Thyroid	.010	.010	.007	.007
Caecum*	1.30	4.08	5.34	1.34
Avg wt of animals, g	39.4	41.6	51.8	49.7

Each figure represents the avg of 5 determinations on individual animals. The data were quite homogeneous.

* Caeca were weighed without removing contents.

† Renal lesions were present in a few of animals on unsupplemented choline-deficient diet on 5th day, and in all of them on 9th day. The data in the table were obtained exclusively from animals which had not yet developed renal lesions.

TABLE IV. Choline in Tissues after 4 Days on Diets.

Diet (5 g/rat/day)	Avg daily wt gain per rat, g	Liver choline, mg/g wet wt	Kidney choline, mg/g wet wt
Part I			
Choline-free	2.8	1.54 1.25	2.13 2.06
" +aureomycin .5%	2.9	1.76 1.86	2.27 2.35
" +choline .3%	3.1	2.74 2.88	2.54 2.45
Part II			
Choline-free	2.5	1.63 1.67	2.02 2.07
" +aureomycin .5%	2.6	1.78 1.89	2.28 2.35
" +choline .3%	3.4	2.70 2.74	2.71 2.51

Results within each bracket represent determinations on duplicate pools of tissue from 5 rats. Determinations in this experiment were done on extracted material without petroleum ether resolution. Several animals on unsupplemented deficient diet had very early renal lesions without significant renal enlargement at the time of the determinations.

observed to contain small quantities of choline, but this might have occurred as a result of contamination of the feces with food. Aureomycin itself gave no reaction for choline when run through the procedures employed in the choline determinations on tissues and feces.

Discussion. Aureomycin has been observed to increase the growth of animals on presumably adequate diets(5), on diets deficient in certain vitamin factors(6), and when administered together with folic acid antagonists(7). This antibiotic also has been noted to decrease dietary hepatic injury in rats(8), and to produce favorable responses in pernicious anemia(9) and fibrocystic disease of the pancreas(10). The flora of the gut is markedly altered by aureomycin(11). Most investigators, while acknowledging the possibility of other mechanisms, have been inclined to attribute these effects of aureomycin to changes in production or utilization of various biologically-active substances in the gut as a result of quantitative or qualitative alterations in the microbial flora.

Since vit. B_{12} appeared to have some protective action under the circumstances of the present experiments, a part of the effectiveness of aureomycin under these circumstances may have been a result of increased production or conservation of vit. B_{12}. Aureomycin has been shown to increase the vit. B_{12} activity of the feces(12), and to decrease mortality on B_{12}-deficient diets(13). The vit. B_{12} activity present in the crystalline aureomycin itself was far too low to produce significant effects, and the choline content was negligible. There is no information available as to whether aureomycin contains labile methyl groups utilizable in the biological formation of choline; it appears unlikely that its protective

activity was based on this mechanism. Studies on liver and kidney choline, and on fecal choline, suggested that an increase in formation or a decrease in destruction of choline, either in the gut or in the tissues, occurred as a result of aureomycin therapy. There is some question, however, of the specificity of the choline method, and there is no assurance that the protective effect of aureomycin was actually a result of the changes in choline content of the tissues. No information was obtained concerning possible decreases in the formation of harmful factors in the gut, or direct effects on tissue metabolism, as a result of aureomycin administration.

Summary. The renal lesions and animal mortality caused by a purified diet deficient in choline were largely prevented by supplementing the diet with rather high levels of crystalline aureomycin. Fatty changes in the liver also appeared to be reduced to some extent. These effects of aureomycin were not due to changes in food consumption, and did not appear to involve the endocrine glands. The effects of aureomycin were not diminished by recrystallization, but destruction of the antibacterial properties by heating in alkaline solution also abolished the protective effects. Studies with diets containing greatly increased amounts of various vitamin factors produced no evidence that the effects of aureomycin might have been due to alterations in production or conservation of any of the factors present in the diet, with the exception of vit. B_{12} and choline itself. The levels of choline in liver and kidney tissue and in feces of deficient animals appeared to be increased slightly as a result of aureomycin administration.

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Presence of Complement in Serum of the Mouse. (19642)

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The results of previous investigations(1-3) have indicated that serum of the mouse is incapable of acting as a source of complement in a hemolytic antigen-antibody combination. The results obtained by Ritz(1) indicated that Endpiece was not present, while Brown (2) concluded that the second component was lacking. Rice and Crowson(3) found that the titers of C'2 and C'4 were less than 10 units per ml of serum. By using fewer sensitized sheep erythrocytes it has been possible to demonstrate complement activity in most mouse sera.

Materials and methods. The Swiss strain of albino mouse, on which all titrations were performed, was obtained from breeding stocks