

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.

B₁₂ 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

TABLE I. Titer of Complement in Individual and Pooled Mouse Sera.

Source		Sex	Units comple- ment/ml serum
Individual	1	♀	2.5
	2	♀	1
	3	♀	1
	4	♀	<1
	5	♀	1
	6	♀	<1
	7	♀	<1
	8	♂	< .5
	9	♂	1
	10	♂	1
	11	♂	.5
	12	♂	1
Pool of 7 mice	7	♂ ♂	1
	7	♂ ♂	1
	2	♀ ♀	1

maintained in this laboratory. Both male and female adult animals were included. Blood was secured either by intracardial puncture or by snipping the brachial artery and withdrawing the blood from the pouch formed by the skin and the body wall. The resulting clot was broken up with a small glass rod and the tube containing blood was centrifuged at 2500 r.p.m. for 15 minutes. The serum was removed and utilized immediately. In order to secure titrations below 10 units per ml of serum, only one tenth the usual numbers of sheep erythrocytes, sensitized with rabbit-anti-sheep-cell amboceptor, were used. Each tube, therefore, contained 0.2 ml of sensitized sheep erythrocytes (approximately 12,500,000 red cells), varying amounts of undiluted mouse serum, and enough saline to make a final volume of 0.8 ml. Magnesium was routinely incorporated into all saline solutions. The tubes were shaken, placed in an incubator and held for 1 hour at a temperature of 37°C. The greatest dilution showing 100% hemolysis was taken as the endpoint and titers were expressed in terms of units per ml of undiluted serum.

Results. In 8 of 12 mice of both sexes,

complement activity was clearly demonstrated (Table I). The highest titer obtained was 2.5 units per ml of serum and in 4 mice there was no discernible activity. The titers of the 3 pooled sera were 1.0 unit per ml each. There was no difference in titer in relation to sex. Heating fresh mouse serum to 56°C for 30 minutes destroyed complementary activity. No natural sheep cell hemolysins could be demonstrated in any mouse serum.

Discussion. The presence or absence of complement activity in a given serum is dependent on the criterion used to define the two terms. The titrations performed by Brown (2) as well as those of Rice and Crowson (3) would measure no less than 10 units of complement per ml of serum. Ritz (1), and Brown (2) concluded that certain components were absent, while Rice and Crowson (3) indicated a deficiency by utilizing the term of "less than 10 units." The earlier investigations were made without the benefit of magnesium in the system. Further studies may reveal yet additional substances to enhance complement activity and thus add further evidence of hemolytic complement activity in sera of animals thought to be deficient. It is perhaps wise, therefore, to question the reported total absence of hemolytic complement in the serum of animals such as the sheep, cow and horse until additional studies utilizing a system which would record titers of lower magnitude than those previously used, have been carried out.

Summary. Using a reduced number of sensitized sheep erythrocytes, hemolytic complement activity in the serums of most mice has been demonstrated.

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