

An Electrophoretic Study of the Serum Proteins of the Rabbit as Influenced by Intravenous Injection of Aureomycin¹

H. C. REITZ and R. A. MESSING, Purdue University

An antibiotic discovered by Duggar in 1948 was named aureomycin because of a yellow pigment formed during the growth of the mold mycelium and because the isolated antibiotic itself was golden yellow in color. On the basis of the chemical structure announced by the Lederle Laboratories in 1952 this antibiotic also has been designated as chlorotetracycline. One of the first of the broad-spectrum antibiotics to be introduced, aureomycin has established its usefulness in the treatment of diseases caused by either gram negative or gram positive bacteria as well as those caused by certain of the large viruses and Rickettsiae. Not only has aureomycin been found of value in the treatment of a wide variety of diseases but it has been shown to have wide usefulness in the field of animal nutrition. It has been shown that the addition of 20 to 40 mgs. of aureomycin per pound of regular diet increases the growth rate of poultry, swine, and livestock by 20 to 40 per cent.

Although aureomycin has such a wide use in medicine and animal nutrition, there appears to be no published data on the effect of intravenously administered aureomycin on the proteins of blood serum. The electrophoretic method of determining the serum proteins was chosen in the present study because of its speed, accuracy, and widespread clinical use in determining blood protein alterations in such diseases as multiple myeloma, portal cirrhosis of the liver, rheumatoid arthritis and a number of others.

Apparatus and Methods

A Perkin-Elmer Model 38 Tiselius Electrophoresis apparatus was used in this investigation. The cell capacity is 2 ml. and the optical system is based on Longsworths (2) scanning modification of the Toepler schlieren method. The model and operational technique are described by Moore and White (4). A barbiturate buffer of 0.1 ionic strength and pH 8.6 was used throughout the study.

To obtain the samples of protein for electrophoretic analysis, the ear of a rabbit was shaved and the skin was rubbed with 90 per cent ethyl alcohol. Then an incision was made in the protruding vein with a razor blade and the blood allowed to drip into a 15 ml. graduated centrifuge tube. To prevent a clot from forming, the incision was frequently wiped with absorbent tissue. When 5 ml. of blood had been collected it was allowed to clot, the red blood cells centrifuged down, and the clear serum removed with a syringe. The serum thus obtained was diluted to three times its volume with barbiturate buffer giving a protein concentration of about two per cent. The resulting protein

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solution was placed in cellophane tubing and allowed to dialyze in a refrigerator, at 5°C. for 20 to 48 hours against one liter of the barbiturate buffer. The period necessary for dialysis was found to be more than 20 hours to reach equilibrium, and less than 48 hours to avoid changes in the relative concentration of the components. After dialysis the sample was introduced into the electrophoresis cell by means of a syringe with a 6-inch needle. The cell was then placed in the ice-water bath of the apparatus. When the solution had reached the temperature of the bath the two halves of the cell were adjusted to form a boundary between the protein solution and the buffer. The voltage was adjusted to give a current of 5.7 to 7.5 milliamperes. A typical electrophoretic pattern for normal rabbit serum taken at the end of two hours is shown in Fig. 1.

Injection of Aureomycin

The sample of aureomycin used in this study was a specially recrystallized aureomycin hydrochloride, lot number 7-7206.² This material was dissolved in distilled water to give a concentration of 15 mg. per ml. The procedure for injection of antibiotic was to shave the ear of the rabbit and rub the vein with alcohol. The hypodermic syringe fitted with a 24 gauge needle and containing the desired amount of aureomycin was then inserted into the protruding vein and the antibiotic allowed to flow slowly into the blood stream of the animal. For the studies at antibiotic levels of 10 to 20 mgs. per kilogram of body weight, the total amount of antibiotic was injected at one time, but for the 40 mgs. per kilogram of body weight level it was found necessary to inject the aureomycin in four equal portions at six hour intervals as several animals died immediately on injection of the entire amount. Although extreme care was taken to avoid contamination and sterile technique was observed throughout in use of syringes and needles, it was noted that the solution of aureomycin seemed to develop toxic properties after 6 weeks even though kept in the refrigerator. It was found that an injection of 20 mgs. per kg. of body weight of the aged solution caused the immediate death of an animal, although the same amount of the freshly prepared antibiotic had previously shown no deleterious effect.

Blood was withdrawn one-half and twelve hours after injection of the antibiotic except in the case of the 40 mg. per kg. of body weight in which the second sample of blood was withdrawn after six and one-half hours. In order to have a valid basis for comparison the electrophoretic pattern for a particular animal was determined a short time before administration of aureomycin to the same animal. The electrophoretic pattern for the serum protein of a rabbit 12 hours after injection of 10 mg. of aureomycin per kg. of body weight is shown in Figure 2. The electrophoretic pattern obtained after injection of the largest amount used is shown in Figure 3.

² We wish to acknowledge our appreciation to Dr. N. Bohonis and the Lederle Laboratories for supplying the aureomycin used in this study.

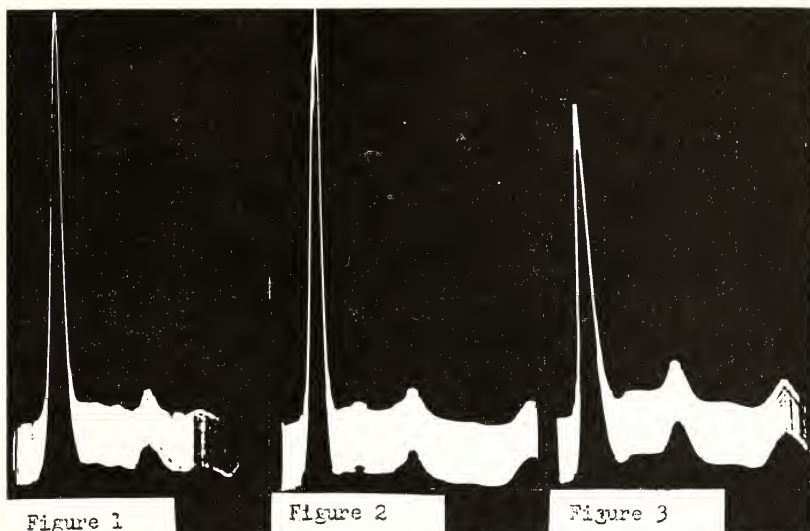


Figure 1. Electrophoresis pattern of normal rabbit serum.

Figure 2. Electrophoresis pattern of rabbit serum 12 hours after injection of 10 mgs. Aureomycin per kg. body weight.

Figure 3. Electrophoresis pattern of rabbit serum 6½ hours after injection of 40 mgs. Aureomycin per kg. body weight.

Calculation of Results

The distance of migration in centimeters of the various fractions was determined by placing the photograph of the initial boundary over the photograph taken at the end of two hours and measuring the distance from the center of the initial boundary to the center of the component in question. Protein mobility, u , was calculated by means of the equation:

$$u = \frac{d K q}{t i}$$

where:

u = protein mobility

d = distance (cm.) migrated

K = conductivity (1/ohms) of buffer

q = cross sectional area of cell - 0.288 cm.²

t = time(sec.)

i = current in amperes

The effect of varying concentration of aureomycin on the mobility of the various components of rabbit blood serum is summarized in Table 1. Values for the corresponding mobilities for the uninjected animal are given for comparison.

The concentrations of the various protein components were calculated by tracing the enlarged electrophoresis patterns on coordinate paper and determining the area under each curve by counting squares. The curves were divided by a straight line at the lowest point for each

TABLE 1

The Effect of Varying Amounts of Intravenously Injected Aureomycin on the Electrophoretic Mobility of Rabbit Serum Proteins

Conc. Injected mg. kg. body weight	Time after last injection, hours	Albumin	Mobility $u \times 10^5$ cm. ² /volt-sec.			
			α_1	α_2	β	γ
Control	Control	6.5	5.1 ¹		3.3	1.5
10	½	6.1	4.8		3.3	1.4
10	12	6.4	5.5	5.0	3.3	1.3
Control	Control	6.1	5.3	4.9	3.1	1.1
20	½	6.1	4.8		3.0	1.2
20	12	6.6	5.7	5.2	3.4	1.3
Control	Control	5.8	4.3	3.7	2.7	1.2
20	½	5.7	4.3	3.6	2.6	1.1
20	12	6.0	4.6	3.9	2.7	1.2
Control	Control	6.0	4.7		3.4	1.5
40	½	6.5	5.1		3.4	1.4
40	6½	6.5	5.1		3.5	1.3

¹ Where only one value is given, the separation of the two components was incomplete.

component according to the suggestion of Longworth (1). Values reported are those obtained from the ascending boundaries.

In Table 2, the values for the concentration of the various protein components in rabbit blood serum as affected by various concentrations and times after injection are presented. Values for the uninjected animal are again given for comparison.

TABLE 2

The Effect of Varying Amounts of Intravenously Injected Aureomycin on the Relative Concentration of Rabbit Serum Proteins

Conc. Injected mg. kg. body weight	Time after last injection, hours	Albumin	Per cent Relative Concentration		
			α	β	γ
Control	Control	63.6	11.4	16.4	8.6
10	½	62.7	10.9	17.5	8.9
10	12	61.4	11.1	17.0	10.5
Control	Control	62.4	14.1	14.4	9.1
20	½	63.7	13.1	14.6	8.6
20	12	64.3	12.7	13.2	9.8
Control	Control	64.0	12.8	13.3	9.9
20	½	61.6	12.9	14.9	7.1
20	12	62.7	14.1	14.1	9.1
Control	Control	63.2	12.1	15.0	9.7
40	½	64.0	13.8	14.6	7.6
40	6½	64.5	11.8	16.8	6.9

Discussion

The average values obtained in the present study for the mobilities of the various components of rabbit blood serum are: albumin 6.2, com-

bined a_1 and $a_2 = 4.9$, $a_1 = 5.0$, $a_2 = 4.4$, $\beta = 3.1$, $\gamma = 1.3$. Moore (3) made a study of species differences in serum proteins using the electrophoretic technique and barbiturate buffer. He reports the following average values for mobilities of rabbit serum proteins: Albumin 6.0 - 6.7, a_1 5.1 - 5.5, a_2 4.2 - 5.0, β 3.1 - 3.2, γ 1.2 - 1.3. A comparison of the two sets of values shows that the results obtained in the present study are well within the range reported by Moore.

The average of all values for the percentage composition of blood serum proteins of normal or uninjected rabbits obtained in this investigation is: Albumin 63.1%, α -globulin 13.0%, β -globulin 14.6%, and γ -globulin 9.1%. This is based on results from 19 animals. Again these values are close to reported by Moore (3): Albumin 63 - 65%, α -globulin 10 - 15%, β -globulin 11 - 13%, and γ -globulin 10-14%. It will be noted that our value for the γ -globulin component is lower than the range reported by Moore. This is thought to be due to the fact that all the rabbits used in this study were 6 months of age or younger while Moore obtained his results from older or mature animals. The possibility exists that the γ -globulins may be formed from the proteins of the β -globulin fraction.

Conclusion

A comparison of the average percentages of the various components in the blood serum of the uninjected animals with corresponding values after intravenous injection of aureomycin indicates that no significant changes have taken place except perhaps a lowering in the γ -globulin fraction one-half hour after injection of the antibiotic. This change is not considered as highly significant of a change in the total pattern since other components do not vary more than 0.5%.

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