

**RATE OF ELIMINATION OF PARENTERALLY ADMINISTERED SERUM
PROTEINS IN RELATION TO THEIR SPECIES ORIGIN**

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It is well known that the effect of immune serum used for therapeutic purposes depends on many factors, such as the specific antibody, the affinity of the antibody for the corresponding antigen and the serum's capacity to be maintained for a long period of time in the recipient's blood stream. A peculiarity of passive immunity is its transience: it remains only as long as the artificially introduced antibodies remain.

It is well known that serum obtained from animals of the same species circulates in the recipient longer than the serum from animals of another species. It has also been noted that the rapidity of elimination of heterologous serum protein of different animals is not the same. Thus, antidipterial antitoxin obtained from horses and horned cattle is eliminated from the rabbit more slowly than antitoxin of human, goat or guinea pig [5]. The causes of the variable length of circulation of parenterally introduced serum proteins have not been studied well, although a number of methods of investigation can be applied toward this end: immunologic, immunochemical, biological, methods for detecting proteins, as well as labeled radioactive compounds, etc. [7-15]. It has been shown that the circulation time of parenterally introduced proteins depends on the site of injection, the concentration of the protein and the previous sensitization of the animal [1, 3, 4, 6, 10, 14].

In the present experimental investigations, an attempt was made to study the relationship of the circulation time of parenterally introduced serum proteins to their species origin. The problem is not only of theoretical interest, but also of practical importance in obtaining more effective therapeutic preparations.

TABLE 1. Circulation Time of Heterologous Serum Proteins in the Blood Stream of the Rabbit (in days)

Serum protein	Days of observation													Mean circulation time of protein, days
	rabbit No.													
	723	856	1836	443	825	824	827	1003	840	822	292	295		
Chicken	4	4	5	5	4	4	4	—	—	2	3	3	}	4
Duck	—	—	—	—	—	—	—	4	3	—	—	—		
Dog	9	13	11	9	9	8	9	11	5	6	9	16		9
Cat	6	9	7	5	22	21	22	7	5	27	9	9		12
Human	16	18	18	9	22	23	25	18	7	23	9	9		16
Pig	18	26	23	27	22	29	25	23	28	34	82	82		34
Horse	21	26	23	27	29	29	25	23	28	39	82	82		36
Sheep	21	26	23	27	29	29	29	23	31	—	—	—		
Bull	—	—	—	—	—	—	—	—	—	39	82	82	}	36

METHOD

The study of the circulation time of serum proteins of animals of different species and of man, as well as the speed of formation of antibody to seven different sera was made by injecting the sera intravenously in the ears of adult chinchilla rabbits weighing 2.5-3 kg. Each rabbit received 1 ml of avian sera (chicken and duck) as well as sera of dog, cat, man, pig, horse, sheep and bull. The sequence of serum injection was varied.

TABLE 2. The Results of the Investigation of Rabbit Serum for the Presence of Precipitins to Heterologous Proteins

Precipitins to serum protein	Days after admini- stration	Titer of precipitin in rabbit serum									
		№ 836	№ 443	№ 825	№ 824	№ 827	№ 1003	№ 840	№ 822	№ 292	№ 295
Chicken or duck	7	100	100	100	—	100	100	1 000	100	5 000	5 000
	9	5 000	1 000	5 000	5 000	1 000	5 000	1 000	1 000	10 000	10 000
	11	5 000	1 000	10 000	5 000	10 000	5 000	1 000	10 000	10 000	10 000
	15	1 000	5 000	10 000	10 000	10 000	10 000	10 000	10 000	10 000	10 000
Bull or sheep	7	100	100	—	—	—	100	100	—	1 000	100
	9	100	100	1 000	—	100	1 000	10 000	5 000	10 000	10 000
	11	10 000	1 000	1 000	5 000	1 000	10 000	10 000	10 000	5 000	—
	15	10 000	1 000	10 000	10 000	10 000	10 000	10 000	10 000	10 000	10 000
Horse	7	100	100	—	—	—	100	1 000	100	100	100
	9	1 000	100	—	100	100	100	10 000	1 000	1 000	5 000
	11	5 000	1 000	1 000	10 000	1 000	1 000	10 000	5 000	10 000	—
	15	5 000	1 000	5 000	5 000	10 000	1 000	10 000	10 000	10 000	10 000
Pig	7	—	100	—	—	—	—	100	100	100	100
	9	100	1 000	—	—	100	1 000	10 000	1 000	10 000	10 000
	11	5 000	1 000	1 000	10 000	100	5 000	10 000	1 000	5 000	—
	15	5 000	5 000	5 000	10 000	1 000	5 000	10 000	5 000	5 000	10 000
Human	7	100	—	—	—	—	100	100	100	100	—
	9	100	100	—	—	—	100	10 000	1 000	5 000	10 000
	11	1 000	1 000	5 000	10 000	1 000	1 000	10 000	5 000	5 000	10 000
	15	10 000	1 000	10 000	10 000	10 000	10 000	10 000	10 000	1 000	—
Cat	7	—	—	—	—	—	—	—	—	1 000	—
	9	100	—	—	—	—	100	1 000	1 000	1 000	10 000
	11	100	—	1 000	—	—	100	1 000	5 000	1 000	—
	15	1 000	1 000	5 000	5 000	5 000	10 000	10 000	5 000	1 000	10 000
Dog	7	100	—	—	—	—	—	—	100	100	100
	9	100	—	—	—	—	100	—	1 000	100	1 000
	11	100	—	—	—	—	100	1 000	1 000	1 000	—
	15	1 000	1 000	10 000	5 000	5 000	10 000	10 000	5 000	1 000	10 000

To observe the fate of injected protein in the rabbit on the day of injected and thereafter (up to complete elimination of all protein) 2-4 ml of blood was taken from the ear of the rabbit. The serum obtained was used as antigen in a precipitin reaction. Specific precipitating serum against human protein and proteins of the dog, cat, swine, horse, sheep and chicken with titers of 1:10,000 were used as antisera. The precipitation reaction was carried out in centrifuge tubes. Rabbit serum was placed in 7 tubes and different heterologous proteins were added (antigen) in a quantity of 0.3-0.4 ml; then the corresponding antiserum was carefully added to each tube with a thin Pasteur pipette. The reaction was considered positive if the precipitin ring appeared in 20 min from the moment of the addition of the serum and was designated "plus." When the ring did not appear in this length of time, the reaction was considered negative and was designated as "minus."

RESULTS

To study the persistence of heterologous protein in the animal, serum was given to 12 rabbits. The data on the length of persistence of foreign protein in the blood of these animals are presented.

It can be seen that the circulation time of each heterologous protein in the blood stream of different rabbits was not the same; thus, the length of persistence in the rabbit of dog protein varied from 5-16 days, of cat protein from 5-27 days, and of human protein from 7-25 days. The circulation time of swine, sheep and horse protein in the rabbit was more uniform.

In the majority of rabbits these proteins were detected for 20-30 days. However, the circulation time of these proteins was significantly greater in 3 rabbits (i.e., in rabbits No. 292 and 295 the aforementioned proteins were detected for 82 days and in rabbit No. 822 for 39 days). The circulation time of avian serum protein in all the rabbits was almost the same and never greater than 5 days.

It is further evident from the data in Table 1 that avian protein disappears from the blood stream of the rabbit significantly more rapidly than the proteins of mammals given at the same time. After avian protein, the protein of mammals was eliminated in succession. The protein of the dog (mean survival, 9 days) and of the cat (mean survival, 12 days) was eliminated earlier than the others. In some rabbits (No. 840 and 292) the dog and cat protein were eliminated at the same time. In the majority of the rabbits the circulation time of these proteins was different; in some (No. 723, 856, 1836, 443, 1003 and 295) a more prolonged circulation of dog protein was observed, in others (No. 825, 824, 827 and 822), of cat protein. The human serum protein disappeared after that of the dog and the cat. The mean length of its circulation was about 16 days. A more prolonged time in all the rabbits was determined to be the fate of the proteins of the pig, horse, sheep and bull. It should be noted that these proteins differed little from each other in their period of persistence in rabbit's blood. Only in some rabbits did the protein of the pig disappear somewhat earlier. In the majority of the rabbits, the serum protein of the pig was detected for 25-30 days. In rabbit No. 822 the protein circulated for 39 days, and in rabbits No. 292 and 295, for 82 days.

Thus, the experiment showed that the serum proteins of different animals and man injected intravenously into rabbits at the same time and in equal concentrations circulate for different lengths of time. The elimination of avian serum protein was significantly more rapid than that of the protein of mammals, which apparently is conditioned by the phylogenetic and antigenic remoteness of these animals; the proteins of the dog and cat were eliminated from the rabbit in a similar length of time and following the elimination of avian proteins human serum protein occupies an intermediate position between the protein of the dog and the cat, on the one hand, and the protein of the pig, sheep and bull and horse, on the other. The similarity in behavior of cat, dog and human serum proteins and their excellence as antigens in comparison with the serum proteins of swine, horse and particularly cattle, was noted by us earlier [2].

The circulation times of heterologous proteins in the blood of the rabbit were compared with data on the speed of formation of specific precipitating antibody to the proteins administered in the blood of the rabbit. The results of the investigation of the rabbit serum for the presence of precipitins to the different proteins on the 7th, 9th, 11th, and 15th day after administration of the serum protein are presented in Table 2.

It can be seen from the data in Table 2 that as early as the 7th day precipitin to avian protein was observed in the blood of all the rabbits in a titer of 1:100 or 1:1000, and, in 2 rabbits of 1:5000, on the same day precipitin to mammalian protein was either absent or was only present in a titer of 1:100 (the least dilution of antigen with which we began to investigate the serum of the rabbits was 1:100, because lesser dilutions could have given a nonspecific reaction.)

On the 9th day after the injection of proteins, specific precipitin to the chicken protein was observed in high titers (1:1000, 1:5000, 1:10,000) in the serum of all the rabbits; precipitins to the sheep, swine and horse proteins

were also found in almost all the rabbits in titers of 1:100-1:10,000. The precipitin titers to human protein were somewhat lower. Only in half of the rabbits was there any antibody to the dog and cat proteins in a titer of 1:100 or 1:1000.

On the 11th to 15th days, precipitins to serum proteins of the chicken and the duck and to the protein of the sheep, bull, pig, horse and man were observed in all the rabbits at high titers. Precipitins to dog and cat proteins reached high titers only on the 15th day.

From comparison of the data on the precipitins to serum proteins and the circulation time of different heterologous proteins, it can be said that there is a relation between the rapidity of disappearance from the blood stream of the rabbit of avian serum proteins and the earlier appearance of high titer of specific precipitins to these proteins in the serum of the rabbit. The proteins of the mammals brought about a different result: despite early appearance in the serum of the rabbit of specific precipitins to the proteins of the pig, bull, sheep and horse, these proteins circulated in the blood of the rabbit the longest time.

Thus, our experiment showed the length of circulation of heterologous proteins administered parenterally to the rabbit could depend on their species origin. The shortest period of survival in the blood of the rabbit was that of avian serum protein (chicken and duck). The serum proteins of mammals in the blood of the rabbit were eliminated later in the order dog, cat and human, followed almost at once by the proteins of the sheep, bull, pig and horse.

The rate of elimination from the rabbit of avian serum protein corresponded to the very early appearance in the blood of the rabbit of specific precipitins to these proteins in high titer. The mammalian proteins could be observed in the blood of the rabbit for longer periods despite the presence of specific precipitins in high titer.

To obtain immune serum which has the greatest circulation time in the blood stream of the recipient, it is necessary to consider the species origin of the animal chosen for immunization.

SUMMARY

One ml each of the serum proteins of chicken (or duck), dog, cat, human, pig, horse, sheep and bull were introduced simultaneously into the blood of 12 rabbits. The fate of the proteins administered was investigated by the precipitin method with the aid of immune specific sera against the proteins named. The investigation showed that avian proteins disappear from rabbit blood much more rapidly than those of mammals, the next to be eliminated are dog and cat proteins, and then come those of humans. The proteins of swine, horse, sheep (or bull) circulate longest in rabbit blood. The earliest precipitins to appear in the rabbit blood were those against avian proteins.

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