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THE COMPARATIVE MERITS OF VARIOUS COMPLEMENTS AND AMBOCEPTORS IN THE SERUM DIAGNOSIS OF SYPHILIS.[1]

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When using a hemolytic antihuman amboceptor produced in the goat, one of us (Noguchi)[2] found that goat complement could not be deviated or fixed by the precipitate formed by mixing an extract of *Diplococcus intracellularis* (Weichselbaum) with antimeningococcic serum (Flexner-Jobling), or by mixing sera of man or different animals with their specific precipitins (rabbit), or egg-white with its specific precipitin. Under similar conditions, goat complement can not be deviated in Noguchi's method of the syphilis reaction. The amboceptor from the goat seemed to have a stronger affinity for the goat complement and liberated the complement after it had been fixed. Noguchi has also repeatedly shown, in the Bordet-Gengou phenomenon or in the syphilis reaction, that rabbit complement is less firmly fixed than guinea pig complement. As he was using a rabbit amboceptor, he could not determine whether the difficulty in fixing this complement was due to the use of an homologous amboceptor (from the same species) or to the inferior fixing qualities of the complement itself. In order to determine this point, it was necessary to prepare amboceptors from different species of animals. The preceding problem, together with others concerning the comparative merits of the complements of various species of animals in the complement fixation phenomenon, the Bordet-Gengou, and the Wassermann reactions, is considered in this communication. The questions just outlined include the determinations of the complementary activity of the sera of various

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species of animals with homologous and heterologous amboceptors. They also bring out the relative capacities of different species of animals for producing hemolytic amboceptor for human erythrocytes. Apart from the scientific interest, we intended also to search for a suitable species of the larger domestic animals whose complement or amboceptor would yield results as reliable as those obtained with guinea pig complement and rabbit amboceptor. If this should be accomplished, an economy would result.

PREPARATION OF ANTIHUMAN AMBOCEPTOR.

In our experiments the washed human erythrocytes suspended in 0.9 per cent. salt solution, corresponding with the original volume of the blood, was used for immunizing the animals. The varieties of animals used and the quantities of erythrocytes injected are given below:

Dog, subcutaneous injection,	20, 30, 30, 30 c.c.
Cat, intraperitoneal injection,	5, 10, 15, 20 c.c.
Rabbit, intraperitoneal injection	5, 10, 15, 20 c.c.
Guinea pig, intraperitoneal injection,	2, 3, 5, 7 c c.
Rat, intraperitoneal injection,	1, 2, 3, 3 c.c.
Chicken, intraperitoneal injection,	3, 4, 5, 5 c c.
Goat,[3] subcutaneous injection.	

With the exception of the goat, more than two animals of each of the species were employed, and several of each of the smaller animals. Injections were made four to five days apart, and the serum was collected on the ninth day after the last injection. These amboceptoric sera were inactivated at 56° C. for thirty minutes. After inactivation, the strength of amboceptor of each of the above animals was determined by titration with homologous and heterologous complement. The complements employed were from dog, cat, rabbit, guinea pig, rat, chicken, goat, pig, sheep, and ox. Owing to the small quantity of blood that could be obtained from the immunized rats, the amboceptor from this animal could not be titrated to the extent of the others.

The amount of complement of each species used was uniformly 0.05 cubic centimeter for each tube. The erythrocytic suspension

[3] This goat was given about twenty subcutaneous injections.

TABLE I.

	Rabbit amboceptor.				Guinea pig amboceptor.				Dog amboceptor.				Cat amboceptor.				Chicken amboceptor.			
	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.	Complete hemolysis.	Moderate hemolysis.	Trace of hemolysis.	No hemolysis.
Rabbit complement.....	**0.005**	**0.003**	**0.0007**	**0.0003**	0.0015	0.0007	0.00035	0.00015	0.0025	0.001	0.0005		0.004	0.0025	0.005					0.05
Guinea pig complement	0.0025	0.0015	0.0005	0.0002	**0.001**	**0.0005**	**0.0002**	**0.0001**	0.025	0.015	0.001	0.0005	0.015	0.01	0.001	0.0005				0.05
Pig complement .	0.04	0.015	0.0025	0.001	0.025	0.015	0.001	0.0005	0.015	0.01	0.0005	0.0002	0.015	0.01	0.001	0.0005	0.08	0.03	0.005	0.002
Sheep complement.....	0.05	0.025	0.005	0.0025	0.05	0.025	0.005	0.0025				0.05				0.05				0.05
Ox complement..	0.045	0.025	0.005		0.04	0.02	0.005		0.04	0.02	0.005		0.025	0.015	0.0035	0.0007				0.05
Rat complement.	0.01	0.005	0.001	0.0005	0.01	0.005	0.0015	0.0005		0.025	0.005	0.002		0.04	0.025	0.015				0.05
Goat complement	0.0025	0.0015	0.0005	0.0002	0.0025	0.001	0.0005	0.0002	0.001	0.0005	0.0002	0.0001	0.005	0.0025	0.0005					0.05
Chicken complement.....		0.07	0.01		0.01	0.005	0.001		0.05	0.03	0.002	0.001	0.07	0.03	0.005	0.002	**0.03**	**0.015**	**0.0005**	
Cat complement	0.0025	0.0015	0.0007	0.0004	0.0025	0.0015	0.0005	0.0002	0.025	0.015	0.0025	0.001	**0.004**	**0.001**	**0.0025**	**0.0005**				0.05
Dog complement.	0.005	0.0025	0.0008	0.0004	0.005	0.0025	0.0005	0.0002	**0.025**	**0.015**	**0.0025**	**0.001**		0.035	0.015	0.01				0.05

TABLE II.

Species of animal furnishing amboceptor.	Arrangement of different complements according to their capacity to reactivate different antihuman amboceptors.								
Rabbit	Amboceptor titer 0.0015 with guinea pig complement goat complement cat complement	Amboceptor titer 0.0025 with dog complement	Amboceptor titer 0.003 with rabbit complement	Amboceptor titer 0.005 with rat complement	Amboceptor titer 0.015 with pig complement	Amboceptor titer 0.025 with ox complement sheep complement	Amboceptor titer 0.07 with chicken complement		
Guinea pig	Amboceptor titer 0.0005 with guinea pig complement	Amboceptor titer 0.0007 with rabbit complement	Amboceptor titer 0.001 with goat complement	Amboceptor titer 0.0015 with cat complement	Amboceptor titer 0.0025 with dog complement	Amboceptor titer 0.005 with chicken complement rat complement	Amboceptor titer 0.015 with pig complement	Amboceptor titer 0.02 with ox complement	Amboceptor titer 0.025 with sheep complement
Dog	Amboceptor titer 0.0005 with goat complement	Amboceptor titer 0.001 with rabbit complement	Amboceptor titer 0.01 with pig complement	Amboceptor titer 0.015 with guinea pig complement dog complement cat complement	Amboceptor titer 0.02 with ox complement	Amboceptor titer 0.025 with rat complement	Amboceptor titer 0.03 with chicken complement	Amboceptor had no action with sheep complement	
Cat	Amboceptor titer 0.001 with cat complement	Amboceptor titer 0.0025 with rabbit complement	Amboceptor titer 0.01 with goat complement	Amboceptor titer 0.015 with guinea pig complement pig complement	Amboceptor titer 0.03 with ox complement	Amboceptor titer 0.035 with chicken complement	Amboceptor titer 0.04 with dog complement rat complement	Amboceptor had no action with sheep complement	
Chicken	Amboceptor titer 0.015 with chicken complement	Amboceptor titer 0.03 with pig complement	Amboceptor had no action with rabbit, guinea pig, sheep, ox, rat, goat, cat, or dog complement.						

was employed in a dose of 0.1 cubic centimeter of a 10 per cent. suspension of washed human corpuscles. The total volume in each tube was made uniformly one cubic centimeter.

The above experiments revealed that a striking relation exists between the various complements and a given kind of amboceptor produced in different species of animals.

This is brought out in Table II.

With the amboceptors produced in guinea pig, cat, and chicken, the strongest action was obtained with their homologous complements. On the other hand, the amboceptors from the dog and rabbit acted more strongly with certain heterologous complements than with homologous complements. It was not the same heterologous complements, however, which showed this peculiarity. The strongest action with rabbit amboceptor was obtained with the complements of guinea pig, goat, cat, and dog. The complementary action of rabbit serum with dog amboceptor was, however, very much stronger than that of the dog complement with the amboceptor obtained from the dog. This dog amboceptor was thirty times more active in the presence of goat complement than in the presence of dog complement. Guinea pig complement, which has hitherto been considered universally suitable, is seen to be inferior to the complements of goat, rabbit, and pig when used with dog amboceptor, and it was without any complementary action for chicken amboceptor. The complements of ox and sheep, which were found to be quite suitable for goat amboceptor, are seen to be less suitable for the amboceptors derived from rabbit, guinea pig, dog, cat, and chicken. It is also remarkable that the sera of all mammals, except the pig, used in our experiments are devoid of any complementary action for chicken amboceptor, while chicken serum contains certain amounts of complement suitable for the amboceptors derived from those mammals.

Thus we have learned that the titers of an amboceptor may vary considerably according to the varieties of sera employed as complement. That the variations revealed in the titration of a given amboceptor are due to the variations in the complement content of the serum used as complement, can be further demonstrated by determining the limit of activity of the sera (serving as complement)

in the presence of a constant amount of amboceptor. In the following table is shown the comparative activity of different sera as complement. Rabbit amboceptor (in excess) was employed in this experiment.

TABLE III.

	Titration of sera as complement with antihuman rabbit amboceptor.									
	Guinea pig complement.	Cat complement.	Goat complement.	Dog complement.	Rabbit complement.	Rat complement.	Pig complement.	Ox complement.	Sheep complement.	Chicken complement.
Quantity for complete hemolysis	0.006	0.006	0.006	0.01	0.015	0.02	0.05	0.07	0.1	
Quantity for moderate hemolysis	0.003	0.003	0.003	0.005	0.007	0.01	0.03	0.035	0.05	0.07
Quantity for trace of hemolysis	0.002	0.002	0.002	0.003	0.004	0.005	0.015	0.015	0.02	0.02
Quantity for no hemolysis	0.001	0.001	0.001	0.0015	0.002	0.003	0.007	0.008	0.01	0.01

Table III shows that the titration of the complementary activity of the sera of guinea pig, cat, and dog is about twice as strong as that of the rabbit for rabbit amboceptor. In this respect sheep serum was about sixteen times weaker, and chicken serum was too weak to produce complete hemolysis.

We have also studied the complementary activity of various sera for the amboceptors derived from heterologous as well as from homologous species. Our results are given in Table IV.

This series of experiments demonstrates three interesting facts: (1) the preference of certain amboceptors for their homologous complements (chicken, guinea pig, goat); (2) the inferiority of certain complements for homologous amboceptors (rat); and (3) the increase of hemolytic activity of complements in general by the use of a larger amount of the amboceptor. The inferiority of homologous complements to certain heterologous complements has already been described with the sera derived from rabbits and dogs (see Table II). That cat amboceptor is more powerful with its homologous complement is shown in the same table. Thus, we find that the amboceptors derived from chicken, guinea pig, goat, and cat are stronger in the presence of their homologous complements,

TABLE IV.

Quantity of complement.	Rabbit complement.		Dog complement.		Cat complement.		Chicken complement.	Pig complement.	Pig complement.	Rat complement.	Guinea pig complement.		Guinea pig complement.		Goat complement.		Goat complement.	
	Rabbit amboceptor.		Dog amboceptor.		Cat amboceptor.		Chicken amboceptor.	Chicken amboceptor	Rat amboceptor.	Rat amboceptor.	Guinea pig amboceptor.		Goat amboceptor.		Guinea pig amboceptor.		Goat amboceptor.	
	(0.025)	(0.0025)	(0.025)	(0.0025)	(0.025)	(0.0025)	(0.1)	(0.05)	(0.1)	(0.1)	(0.025)	(0.002)	(0.03)	(0.005)	(0.025)	(0.0025)	(0.03)	(0.005)
0,1	C.H.	C.H.	Mch.H.	Mch.H.	C.H.	C.H.	C.H.	C.H.	C.H.	Sl.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.
0.05	C.H.	Md.H.	Tr.H.	Tr.H.	C.H.	C.H.	C.H.	Mch.H.	C.H.	No H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.
0.035	C.H.	Sl.H.	Tr.H.	No H.	C.H.	Sl.H.	Sl.H.	Tr.H.	C.H.		C.H.	C.H.	C.H.	Tr.H.	C.H.	C.H.	C.H.	C.H.
0.02	C.H.	Tr.H.	No H.		C.H.	No H.	No H.	No H.	Mch.H.		C.H.	No H.	C.H.	No H.	C.H.	Md.H.	C.H.	C.H.
0.01	C.H.	Tr.H.			Mch.H.				Tr.H.		C.H.		Mch.H.		Mch.H.	No H.	C.H.	Sl.H.
0.005	C.H.	No H.			Tr.H.				No H.		C.H.		Sl.H.		No H.		Md.H.	No H.
0.002	Md.H.				No H.						Mch.H.		Tr.H.				Tr.H.	
0.001	No H.										No H.		No H.				No H.	

C.H. = complete hemolysis; Mch.H. = much hemolysis; Md.H. = moderate hemolysis; Sl.H. = slight hemolysis; Tr.H. = trace of hemolysis.

while those from rabbit, dog, and rat are stronger in the presence of certain suitable heterologous complements.

After studying the complex inter-relation of various complements and amboceptors, we took up the further study of a few complements. The sera of pig, sheep, and ox were studied especially because we hoped to be able to utilize them as complement, for they may be obtained in large quantity from any abattoir. We soon discovered, however, that they were quite unsuitable for the syphilis reaction. In the first place, ox serum often contains a variable and sometimes considerable amount of natural amboceptor for human erythrocytes, and for this reason we did not pursue the study of this serum.

The sera of pig and sheep are usually devoid of the natural anti-human amboceptor. They were examined for their complementary activity for rabbit amboceptor. The results are shown in Tables V and VI.

The striking feature of the sera of these two animals is the rapid disappearance of their complementary activity. It is more marked with pig serum. Both varieties of serum became so weak after forty-eight hours (0° C.) that they no longer produced complete hemolysis in a quantity as large as 0.1 cubic centimeter. Guinea pig serum, under the same conditions, showed practically no diminution during the same period of preservation. The rapid deterioration of both pig and sheep serum renders them unreliable because of the constantly decreasing activity of complement. The rate of deterioration is also quite irregular with different samples of the same variety of serum. Thus, in point of reliability and activity, they are far inferior to the serum of the guinea pig and are not recommended for use in the syphilis reaction.

CONCERNING THE USE OF VARIOUS SERA AS COMPLEMENT IN THE SERUM REACTION OF SYPHILIS.

In deciding whether or not the serum of a given animal is suitable as complement in the complement deviation test, we have to consider two essential properties of the serum. The first is, of course, its complementary activity, and the second is its susceptibility to fixation. We have already studied in detail the comparative com-

TABLE V.

Pig Serum.

Serial number of pigs.	Titration with antihuman rabbit amboceptor, 0.01 c.c.									Titrated for natural antihuman amboceptor.	
	4 hours old.			24 hours old.			48 hours old.			4 hours old. 0.2	Inactivated at 56° C. 0.2
	0.1	0.05	0.025	0.1	0.05	0.025	0.1	0.05	0.025	Active.	+ 0.05 guinea pig serum.
No. 1.....	C.H.	C.H.	C.H.	C.H.	Mch.H.	Sl.H.	Sl.H.	Tr.H.	No H.	No H.	No H.
No. 2.....	C.H.	C.H.	C.H.	C.H.	C.H.	Mch.H.	Sl.H.	Tr.H.	No H.	No H.	No H.
No. 3.....	C.H.	C.H.	C.H.	C.H.	C.H.	Mch.H.	Md.H.	Tr.H.	No H.	No H.	No H.
No. 4.....	C.H.	C.H.	C.H.	C.H.	Mch.H.	Sl.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 5..... ..	C.H.	C.H.	C.H.	C.H.	C.H.	Mch.H.	Mch.H.	Tr.H.	No H.	No H.	No H.
No. 6.....	C.H.	C.H.	C.H.	C.H.	C.H.	Sl.H.	Mch.H.	Tr.H.	No H.	No H.	No H.
No. 7.....	C.H.	C.H.	C.H.	C.H.	Mch.H.	Mch.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 8.....	C.H.	C.H.	C.H.	C.H.	Mch.H.	Sl.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 9.....	C.H.	C.H.	C.H.	Mch.H.	Mch.H.	Sl.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 10....	C.H.	C.H.	C.H.	C.H.	Sl.H.	Tr.H.	Sl.H.	No H.	No H.	No H.	No H.
Guinea pig serum. (control)	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.		

TABLE VI.

Sheep Serum.

Serial number of sheep.	Titration with antihuman rabbit amboceptor, 0.01 c.c.									Titrated for natural antihuman amboceptor.	
	4 hours old.			24 hours old.			48 hours old.			4 hours old. 0.2	Inactivated at 56° C. 0.2
	0.1	0.05	0.025	0.1	0.05	0.025	0.1	0.05	0.025	Active.	+ 0.05 guinea pig serum.
No. 1	C.H.	Almost C.H.	Mch.H.	C.H.	Mch.H.	No H.	Mch.H.	Tr.H.	No H.	No H.	Tr.H.
No. 2	C.H.	Almost C.H.	Mch.H.	Almost C.H.	Tr.H.	No H.	Sl.H.	No H.	No H.	No H.	No H.
No. 3	C.H.	Almost C.H.	Mch.H.	C.H.	Sl.H.	No H.	Almost C.H.	No H.	No H.	No H.	No H.
No. 4	C.H.	C.H.	Mch.H.	C.H.	Almost C.H.	Sl.H.	Mch.H.	Sl.H.	Tr.H.	Tr.H.	Sl.H.
No. 5	C.H.	C.H.	Mch.H.	C.H.	Almost C.H.	No H.	C.H.	Tr.H.	No H.	No H.	No H.
No. 6	C H.	C.H.	Mch.H.	Almost C.H.	Almost C.H.	Tr.H.	Sl.H.	No H.	No H.	No H.	No H.
No. 7	C.H.	C.H.	Mch.H.	C.H.	Mch.H.	No H.	Md.H.	Tr.H.	No H.	No H.	Tr.H.
No. 8	C.H.	C.H.	Mch.H.	C.H.	Almost C.H.	Tr.H.	C.H.	Almost C.H.	Tr.H.	Tr.H.	Sl.H.
Guinea pig serum (control)	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.	C.H.		

plementary activities of the sera of various species of animals. We propose to report here, therefore, the results which we have obtained with regard to their comparative fixability.

As mentioned at the beginning of this article, Noguchi long ago showed that the complement of goat serum remains undeviated by specific precipitates or by the syphilis reaction. This phenomenon was decidedly more pronounced when the goat furnished both complement and amboceptor. He has also demonstrated that rabbit serum is less sensitive to fixation than guinea pig serum. Hence he suspected that the amboceptor might have such a strong affinity toward its homologous complement as to reverse partially the process of fixation. The question of whether or not there is such a relation between the source of amboceptor and the fixability of complements was left unsettled. This was one of the problems which we set out to solve, and our results will now be given.

The fixation test was carried out with Noguchi's system in the usual way. The positive serum was from a case of untreated secondary syphilis, and, in the quantity of 0.002 cubic centimeter, was capable of fixing completely 0.04 cubic centimeter of guinea pig serum. In the following experiments, 0.02 cubic centimeter of the syphilitic serum was used for each tube.

TABLE VII.

	Complement.				
	Guinea pig 0.04 (2 units).	Rabbit No. 1 0.08 (2 units).	Rabbit No. 2 0.08 (2 units).	Goat No. 1 0.04 (2 units).	Goat No. 2 0.04 (2 units).
Guinea pig amboceptor (2 units)	No hemolysis	Partial hemolysis (no hemolysis for some time).	Partial hemolysis (no hemolysis for some time).	Complete hemolysis (much delay).	Complete hemolysis (much delay).
Rabbit amboceptor (2 units).	No hemolysis	Partial hemolysis (no hemolysis for some time).	Partial hemolysis (no hemolysis for some time).	Complete hemolysis (much delay).	Complete hemolysis (much delay).
Goat amboceptor (2 units).	Slight hemolysis (no hemolysis for some hours).	Much hemolysis (no hemolysis for some time).	Much hemolysis (no hemolysis for some time).	Complete hemolysis (slight delay).	Complete hemolysis (slight delay).

The experiments given above show that the complement contained in goat serum is totally unfixable. There was some delay in commencing hemolysis, but it was finally completed within a few hours. The complement contained in rabbit serum showed the peculiarity that hemolysis commenced after six hours or so, and after about twenty-four hours a moderate amount of hemolysis was observed. This is most striking when compared with the complete and lasting inhibition of hemolysis in the case of guinea pig complement. The use of goat amboceptor, however, has caused more or less hemolysis even with guinea pig complement. Experiments also show that when the complements are fixable the homologous amboceptor and complements do not influence the fixation phenomenon. It is difficult to explain why the fixation of guinea pig complement is imperfect when tested by goat amboceptor. It may be due to the presence in guinea pig serum of a non-fixable complement, which, while quite active with the goat amboceptor, is inactive with the amboceptors from rabbit and guinea pig. Now, with regard to the state of non-fixation of the complement of goat serum, there are two possibilities. The first is its insensitiveness to fixation. The delay in commencing hemolysis may be accounted for by assuming that the quantity of complement still free in the mixture was considerably reduced by a partial fixation. The second is the gradual liberation of complement already fixed through the introduction of the goat amboceptor. Apart from these hypothetical considerations, the above experiments showed that rabbit and goat complement and goat amboceptor are unsuitable in deviation tests.

We have determined the fixability of the complements contained in the sera of pigs and sheep. Pig complement was fixable, while that of sheep was somewhat less so. But, as has already been pointed out, their complementary activity is so feeble that, in order to employ two units, their absolute quantities may reach 0.1 cubic centimeter or even exceed this. If the fixation were *selectively* directed to the complement, an accurate result could always be secured by employing any quantity in which two activity-units existed. But as we shall describe elsewhere, the fixation is not selectively directed to the complement. The presence of non-complementary protein constituents may, therefore, interfere with the

fixation of complement. From this it will be easy to understand why we must avoid weak sera, for in these the quantity which contains a single unit of complement is too large.

CONCLUSIONS.

1. The maximum activity of an antihuman hemolytic amboceptor may be obtained by employing the homologous or heterologous complement, according to the variable relations existing between the species furnishing the amboceptor and the one supplying the complement. Thus, some amboceptors are best reactivated by the complement of the same species, while others may act most strongly when reactivated with the complements of certain suitable heterologous species.

2. From the above it is clear that the complementary activity of a given serum may be very variable according to the varieties of amboceptors employed. In expressing the complementary activity of a serum, the species of the host of the amboceptor must always be stated. Thus, one serum may have many different complementary titers according to the amboceptors used. A similar variation in the titers of the amboceptors occurs when a variety of complements are employed.

3. Certain species of animals (pig and sheep) yield sera which are comparatively poor in reactivating most varieties of antihuman amboceptors. The complements of these species deteriorate rapidly.

4. The serum of chicken contained but little complement for the amboceptors derived from the mammalia, while the amboceptor from the chicken was only poorly, or not at all, reactivable by the complements contained in the mammalian sera. The serum of pig was the only variety which reactivated this amboceptor in a fair degree.

5. For the fixation tests guinea pig complement is the most favorable. This complement is also the most active and durable of those which have been studied. The complements of pig and sheep are quite fixable, but their weakness and rapid deterioration render them unsuitable for fixation purposes. Rabbit complement is quite active but is not easily fixable. Goat complement is, as already

stated, difficult to fix, in spite of its strong complementary activity. The other complements are unsuitable because of their feeble complementary activity.

6. For fixation tests the antihuman amboceptors produced in the rabbit and guinea pig are suitable. They are, moreover, very active and do not cause the phenomenon of non-fixation. The amboceptors from other animals are unsuitable, as we cannot find a complement which strongly reactivates them. The amboceptor from the goat is unsuitable because of the danger of masking the fixation phenomenon by subsequent hemolysis.

7. In summing up, we arrive at the conclusion that the rabbit is the best animal for producing antihuman amboceptor, and the guinea pig for supplying complement. The guinea pig produces a good amboceptor, but its small size renders it second in choice.

So far, no other animals have been found useful for the fixation experiments.