

SEROLOGICAL STUDIES ON THE BLOOD OF THE
PRIMATES.

III. DISTRIBUTION OF SEROLOGICAL FACTORS RELATED
TO HUMAN ISOAGGLUTINOGENS IN THE BLOOD OF
LOWER MONKEYS.

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It has been shown that agglutinins exist in the sera of various animals, which react on the isoagglutinogens of human erythrocytes, and conversely that there are substances in animal blood cells resembling the human isoagglutinogens.¹ Von Dungern and Hirschfeld found that treatment of normal human serum with the erythrocytes of certain animals, for example the rabbit and the steer, remove a part of the isoagglutinins. Blood cells of other species fail to do this. The factors in the corpuscles which were responsible for the absorption do not completely resemble those in human blood. This is shown by the fact, for instance, that substances are present in rabbit serum which act upon the human agglutinable factor B,² but which, as is to be expected, do not react on rabbit blood.

Related findings on the similarity of the factor A to the Forssman antigen have been made by Schiff and Adelsberger.³

¹ Landsteiner, K., *Wien. klin. Rundschau*, 1902, No. 40. von Dungern and Hirschfeld, *Z. Immunitätsforsch., Orig.*, 1910-11, viii, 526. Hooker, S. B., and Anderson, L. M., *J. Immunol.*, 1921, vi, 419. Halpern, J., *Z. Immunitätsforsch., Orig.*, 1911, xi, 609.

² We designate the isoagglutinable factors of human erythrocytes by the usual symbols: A for that in Group II cells; B for that in Group III cells.

³ Schiff, F., and Adelsberger, L., *Centr. Bakt., 1. Abt., Orig.*, 1924, xciii, 172; *Z. Immunitätsforsch.*, 1924, xl, 335. Hesser, S., *Acta med. Scand.*, 1924, suppl. 9. Amzel, R., Halber, W., and Hirschfeld, L., *Z. Immunitätsforsch.*, 1925, xlii, 369.

We have undertaken to search for group factors in the bloods of the lower monkeys for two reasons; first, in order to compare the findings with those obtained with the bloods of the anthropoid apes and already reported;⁴ secondly, because we considered it desirable to determine by examination of a number of species of certain families of animals whether the presence of the serological factors in the blood cells of animals bears any general relationship to their position in the systematic zoological classification.

It has been pointed out in preceding papers⁴⁻⁷ that the relationships thus far established between the zoological classification of animals and their serological qualities differ for proteins on the one hand and the agglutinogens and lysinogens of cells, especially erythrocytes, on the other. The relationship is not as evident in the latter case as in the former, the zoological distribution of the serological factors appearing rather irregular. Yet it does not follow that laws of some kind will not be found.

EXPERIMENTAL.

Methods.—The absorption method was not suitable for our purpose because we often had at disposal only small quantities of blood. This method has the additional disadvantage of measuring only the gross differences between treated and untreated sera, not giving clear-cut results when but a small fraction of the antibody is absorbed. We have, for these reasons, employed the technique used in the experiments on the anthropoid apes; namely, agglutination tests by means of "purified agglutinin solutions," absorbed group-specific immune sera, and a heterogenetic (Forssman) antiserum. These methods are described as Methods B, C, and D in the preceding communication.⁴

In Table I are given some representative tests on monkey bloods. The following list contains the species examined, arranged according to families and genera. The number of individual members of each species tested appears in parentheses whenever that number exceeds unity.

⁴ Landsteiner, K., and Miller, C. P., Jr., *J. Exp. Med.*, 1925, xlii, 853.

⁵ Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1924, xl, 91.

⁶ Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1925, xlii, 123.

⁷ Landsteiner, K., and Miller, C. P., Jr., *J. Exp. Med.*, 1925, xlii, 841.

TABLE I.
Agglutination Tests on Monkey Erythrocytes.*

The agglutinin solutions were prepared from human Group II and Group III sera according to Method B. The immune sera were group-specific, anti-human, erythrocyte sera (Group II and Group III), which had been absorbed with human Group I cells according to Method C. Serum 54 was a heterogenetic (Forsman) antiserum prepared by the immunization of rabbits with horse kidney (Method D).

	<i>Macacus rhesus</i> .	<i>Macacus rhesus</i> .	Mangabey.	Moustached monkey.	Pig-tailed monkey.	Bonnet monkey.	Green monkey.	Vervet monkey.	Sapajou.	Sapajou.	Woolly monkey.	Howler.	Lemur.	Chimpanzee (Group II).	Human (Group II).	Human (Group III).
Agglutinin solution from human Group II serum.....	0	F. tr.	0	0	0	0	0	0	+	+	+	+	+	0	0	+
Agglutinin solution from human Group III serum.....	0	0	0	0	0	0	0	0	Tr.	Tr.	0	0	F. tr.	+	+	0
Absorbed immune serum for Group II.....	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0
“ “ “ III.....	0	0	0	0	0	0	0	0	Tr.	Tr.	0	0	0	0	0	±
“ “ “ 54 (heterogenetic).....	Tr.	F. tr.	0	0	0	0	±	+	0	0	Tr.	+	+	+	+	±

Readings after 2 hours at room temperature.

* In the tables the following signs are used: 0 = negative; F. tr. = faint trace of agglutination; Tr. = trace; ±, +, ++, etc.

List of Monkeys.

	No. examined.
I. Old World Monkeys. Cercopitheciada.	
Baboons—Genus <i>Papio</i> .	
Guinea baboon.....	<i>P. papio</i> or <i>P. sphinx</i> . (3)
Mantled “	<i>P. hamadryas</i> .
Species uncertain; either <i>P. porcarius</i> or <i>P. cynocephalus</i> .	(4)
Guenon Monkeys. Genus <i>Cercopithecus</i> .	
Green monkey.....	<i>C. sabæus</i> . (2)
Vervet “	<i>C. pygerythrus</i> . (3)
Patas “	<i>C. patas</i> .
Mona “	<i>C. mona</i> . (3)
Moustached “	<i>C. cephus</i> .
Hochuer “	<i>C. nictitans</i> .
Mangabeys. Genus <i>Cercocebus</i> .	
Sooty mangabey.....	<i>C. fuliginosus</i> . (2)
White-collared mangabey.....	<i>C. collaris</i> .
Gray-cheeked “	<i>C. albigena</i> .
Macaque Monkeys. Genus <i>Macacus</i> .	
<i>M. rhesus</i>	(16)
Java monkey.....	<i>M. cynomolgus</i> . (2)
Lion-tailed monkey.....	<i>M. silenus</i> .
Bonnet monkey.....	<i>M. sinicus</i> . (2)
Pig-tailed monkey.....	<i>M. nemestrinus</i> .
Black macaque.....	<i>M. maurus</i> .
II. New World Monkeys. Platyrrhina.	
Family Cebida.	
Sapajous, or Capuchin Monkeys. Genus <i>Cebus</i> .	
Brown sapajou	<i>C. fatuellus</i> . (2)
Weeper “	<i>C. capucinus</i> . } (5)
White-fronted sapajou.....	<i>C. albifrons</i> . }
White-throated “	<i>C. hypoleucus</i> .
Black-footed <i>Cebus</i>	
Woolly Monkeys. Genus <i>Lagothrix</i> .	
Humboldt's woolly monkey.....	<i>L. lagotrica</i> . (3)
Spider Monkeys. Genus <i>Ateles</i> .	
Black spider monkey.....	<i>A. ater</i> . (3)
Black-headed spider monkey.....	<i>A. cucullatus</i> .
Howling Monkeys. Genus <i>Mycetes</i> .	
Red howler.....	<i>M. seniculus</i> .
Squirrel Monkeys. Genus <i>Chrysothrix</i> .	
<i>Chrysothrix</i> (species uncertain; probably <i>sciurea</i>).	
Douroucolis. Genus <i>Nyctipithecus</i> .	
Three-banded douroucoli.....	<i>N. trivirgatus</i> .
Family Hapalida.	
Marmosets.	
Common marmoset.....	<i>Hapale jacchus</i> . (3)

Lemurs.

Family Lemuridæ, of the suborder Lemuroidea.

True Lemurs. Genus *Lemur*.

Mongoose lemur.....	<i>L. mungos.</i>	(2)
White-fronted lemur.....	<i>L. albifrons.</i>	
Black lemur.....	<i>L. macaco.</i>	(2)
Ring-tailed lemur.....	<i>L. catta.</i>	
Red-brown ".....	<i>L. rufifrons.</i>	
Spotted lemur.....		

In addition the bloods of two mandrills (*Papio maimon*) and one drill (*Papio leucophæus*) were obtained, but they could not be satisfactorily examined because for unknown reasons spontaneous clumping of the cells occurred in suspensions of the washed cells in saline.

A total of 76 individuals of 36 species has been examined with the following results:

The 46 individuals of the 18 species of Old World monkeys of the family Cercopithecidæ (Catarrhina) gave, in almost all instances, entirely negative reactions with the agglutinin solutions prepared from normal human Group II and Group III serum. The rare faint reactions which occurred may or may not have been due to imperfections in the technique.

In striking contrast to the results with the Cercopithecidæ were those obtained with the New World monkeys (Platyrrhina). All of the 22 individuals of the 12 species examined gave strongly, or at least distinctly, positive reactions⁸ with the agglutinin solution Group II. With the absorbed group-specific immune serum they gave either negative or very weak reactions, which latter, in our opinion, are not due to group-specific antibodies, but rather to the heteroagglutinins present in the (rabbit) sera.

In the same manner the bloods of some members of the family of lemurs (Lemuroidea), of which 8 individuals of 6 species were examined, reacted positively with the Group II agglutinin solution.

The blood of 3 or 4 species of lemurs examined reacted positively with Forssman antiserum. The agglutination of 2 of these bloods by Group II immune serum may be connected with this reaction. In one case which was negative with Forss-

⁸ Only a weak reaction was obtained with one blood of douroucoli (*Nyctipithecus trivirgatus*), not contained in the list, the only instance in which blood of a dead animal was used.

man antiserum a moderate agglutination took place with Group III immune serum. The significance of this reaction could not be determined by absorption tests because of lack of material.

For comparison absorption experiments were carried out in some cases. The results were in complete agreement with those obtained with our routine method. An example is given in Table II, which shows that the blood of the two *Platyrrhina* (sapajous) absorbed almost the whole agglutinin content of the normal human Group II

TABLE II.

Absorption of Normal Human Serum by Monkey Bloods.

First absorption: Normal human serum (Group II) absorbed for 1 hour at room temperature and overnight in the ice box with one-half volume of packed, washed sediment of each monkey blood. Tests made as follows:

- 0.05 cc. absorbed serum.
- 0.1 cc. salt solution.
- 0.1 cc. 2.5 per cent suspension of human Group III erythrocytes.

Second absorption: Supernatant absorbed again for 1 hour at room temperature with one-fourth volume of packed sediment of each monkey blood. Tests made as follows:

- 0.1 cc. absorbed serum.
- 0.1 cc. salt solution.
- 0.1 cc. 2.5 per cent suspension of human Group III erythrocytes.

Human serum Group II.	Erythrocytes used for absorption.				
	Macaque.	Baboon.	Sapajou.	Sapajou.	
Before absorption.....					+±
After 1st absorption.....	+±	+±	F.tr.	F.tr.	
“ 2nd “	+±	+±	Tr.	F.tr.	

serum, while the blood of the two *Cercopithecidae* (baboon and macaque) failed to diminish the agglutinin reaction.

Titration of the agglutinin solution for Group II against one blood each of the *Cercopithecidae* and *Platyrrhina* (macaque and sapajou) showed that in the case of the former no reaction occurred with 6 drops of the solution, while the smallest quantity which still agglutinated the latter was 1 drop of a 1/8 dilution.

In addition to the tests on monkeys the bloods of other animals have been examined in the same manner as the monkey bloods. Some examples of these tests are given in Table III.

TABLE III.
Agglutination Tests on Erythrocytes of Other Animals.

For methods see the legend to Table I.

	Mouse.	Rabbit.	Rabbit.	Rat.	Guinea pig.	Duck.	Pigeon.	Goose.	Donkey.	Horse.	Pony.	Goat.	Cat.	Dog.	Human II.	Human III.
Agglutinin solution from human Group II serum.....	+	+	±	±	+	+	0	0	+	0	0	0	+	±	0	+
Agglutinin solution from human Group III serum.....	0	Tr.?	+	Tr.	0	0	0	0	0	0	0	0	0	Tr.?	+	0
Absorbed immune serum for Group II.....	0	0	0	Tr.	0	0	0	±	0	0	0	0	0	Tr.	+	0
" " " III.....	0	0	0	0	0	+	0	Tr.	0	0	0	0	0	0	0	±

Readings after 2 hours at room temperature.

Tests with the agglutinin solution prepared from human Group II serum gave distinctly positive reactions on the bloods of rabbit, guinea pig, rat, mouse, cat, dog, steer, donkey, and pig, and negative reactions on sheep, horse, goat, pigeon, chicken, duck, and goose bloods. The Group III agglutinin solution gave positive reactions only on the bloods of rabbit and sheep, and negative reactions on those of all the rest. The occurrence in this series of a considerable number of positive reactions with the Group II agglutinin solution is in accordance with the findings of von Dungern and Hirschfeld already mentioned. These authors found distinct individual differences by means of absorption experiments, but in a limited number of such tests we found either no difference, or, at the most, only slight ones.

DISCUSSION.

It follows from the results described that, as has been previously shown, the isoagglutinins of normal human serum act upon the red blood cells of a considerable number of animals, and the conclusion may therefore be drawn that serological substances similar to the human isoagglutinogens have a widespread distribution in the animal kingdom. But it has also been demonstrated by means of immune sera⁹ from rabbits that the structures in the human and animal erythrocytes, though similar, are not identical. For the absorbed group-specific immune sera failed to give any definite reactions with the animal erythrocytes susceptible to the action of normal human isoagglutinins. In our studies only the blood of the anthropoid apes gave such reactions, a fact attesting to the close relationship with man.¹⁰

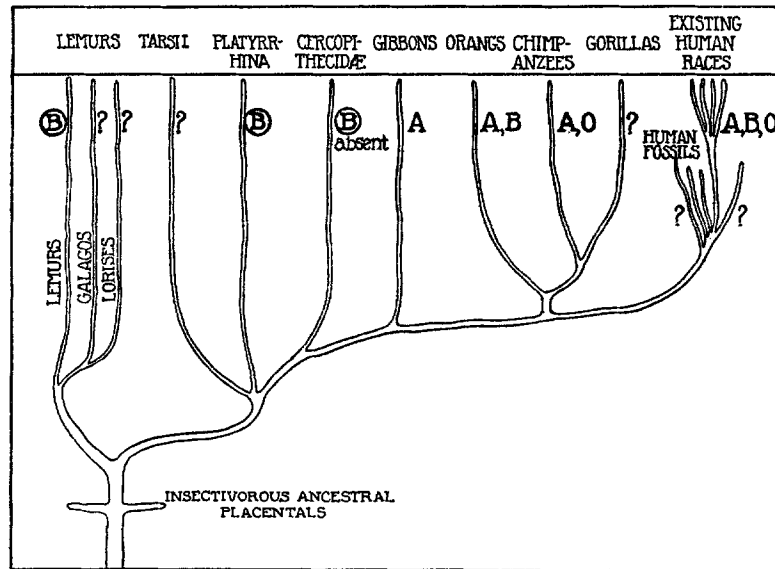
The bloods of ducks and geese reacted with both Group II and Group III immune sera, the former moderately, the latter slightly. But these reactions of duck blood were found to be due to heteroagglutinins which did not correspond to the group agglutinins, for the reactions persisted after treatment of the sera with human Group II and Group III cells. No test of the sort was made with goose blood.

The investigation of the bloods of the lower monkeys brought out an unexpected regularity in the distribution of agglutinogens. Up to the present the zoological distribution of the few agglutinable factors

⁹ Sera obtained by the immunization of chickens behaved in a different way. The problem presented by this difference will not be considered here.

¹⁰ It is possible though not probable that the one case mentioned above of a lemur constitutes an exception.

studied by previous workers has, on the whole, appeared to be quite irregular. To be sure, the factor related to B had been found only in mammals, not in birds, but a very small number of species of the latter



TEXT-FIG. 1. Adapted from Keith, A., *The antiquity of man*, London, 2nd edition, 1925, and Sonntag, Ch. F., *The morphology and evolution of the apes and man*, London, 1924.

A = agglutinin of human Group II red cells.

B = agglutinin of human Group III red cells.

Encircled B = agglutinin similar to, but not identical with, B.

O = blood and serum corresponding to human Group I.

? = not examined.

Of the gibbons only one individual was examined.

have been examined. Also the Forssman antigen is usually regarded as exemplifying the irregularity of the distribution of receptors.¹¹

¹¹ Possibly a law is indicated by the fact that some very closely related species are similar in that the Forssman antigen is present in their blood (Schmidt, H., *Die heterogenetischen Hammelblutantikörper und ihre Antigene*, Leipzig, 1924). In this connection it may be mentioned that a positive agglutination was obtained with the blood of three species of lemurs tested with Forssman antiserum prepared by the injection of horse kidney.

In view of these facts it is noteworthy that we have found by our studies in monkeys that whole genera or families appear to be characterized by the presence of certain serological factors (so called receptors) in their erythrocytes. A factor similar to B was found in all of the bloods of the family Platyrrhina examined (genera *Cebus*, *Lagothrix*, *Ateles*, *Myceles*, *Chrysothrix*, *Nyctipithecus*), in the family of the marmosets (Hapalidæ), and also in the family of lemurs (Lemuridæ). This factor was not found in the blood of the Cercopithecidæ, of which the following genera were examined (*Papio*, *Cercopithecus*, *Cercocebus*, and *Macacus*). While it is obviously impossible to maintain that exceptions may not be found subsequently the uniformity of our results thus far would seem indicative of a definite rule. A peculiarity of the phenomenon unlike the phenomena of specificity in precipitin reactions on serum proteins is the lack of a gradual transition from one family to another—its sharp discontinuity. This point is evident from Text-fig. 1 in which the findings on the group factors in primates are presented.

The results of our studies should stimulate further investigation in the same general direction since it is possible that like zoological relations exist in the case of other serological factors. The human isoagglutinin lends itself readily to the search for certain agglutinogens. It is possible that with methods resembling ours the zoological distribution of other serological factors can be investigated.

SUMMARY.

Serological studies on the bloods of thirty-six species of lower monkeys have shown that there exists a correspondence between the distribution of a certain hemagglutinin and the place of the species in the zoological system.

In twelve species of seven genera of Platyrrhina (New World monkeys) and six species of the genus *Lemur* a factor similar to the human isoagglutinin B was present; in eighteen species of four genera of Cercopithecidæ (Old World monkeys) it was absent, although the latter are more closely related to man than the former.

It would seem from our findings that a genus, perhaps even a family, of animals may be characterized by a special serological factor. The factor found in the lower monkeys is not identical with the one existing in the erythrocytes of the anthropoid apes and man.