SEROLOGICAL STUDIES ON THE BLOOD OF THE PRIMATES.*

I. The Differentiation of Human and Anthropoid Bloods.

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The problem of man's kinship to his closest relatives in the animal kingdom has been studied by the methods of comparative anatomy and paleontology and the results of these investigations are developing into a special branch of learning. When serology provided a new technique for recognizing the biochemical properties which characterize species it became inevitable that this technique should be applied to the problem of man's ancestry.

The serological procedure which has been chiefly followed is that of the precipitin reaction.

Grünbaum[1] made the first experiments with anthropoid blood. He found that anti-human serum gave a precipitate with the blood of the gorilla, the orang-utang, and the chimpanzee "practically indistinguishable from that obtained with human blood either in quality or quantity. Occasionally it has seemed that the blood of the orang gave a more gelatinous precipitate as compared with the granular precipitate of the other bloods, but this may have been due to accidental circumstances." He prepared immune sera against gorilla, orang, and chimpanzee bloods and was "unable to assert that there is any difference of reaction amongst the many combinations of anti-serum and blood which can be made with the four above-mentioned bloods and sera." In consideration of these conclusions it seems difficult to interpret the apparently specific reactions observed by him microscopically and mentioned incidentally.

The well known studies of Nuttall[2] on the precipitin reaction had for their object the correlation of the serological properties of the serum protein of

* A preliminary communication has appeared in *Science* (Landsteiner, K., and Miller, C. P., Jr., *Science*, 1925, lxi, 492).

[1] Grünbaum, A. S. F., *Lancet*, 1902, i, 143.

[2] Nuttall, G. H. F., Blood immunity and blood relationship, Cambridge, 1904.

animals with their position in the zoological system. Using several anti-human sera and one each of anti-chimpanzee, anti-orang, and anti-baboon serum, Nuttall made qualitative tests on the serum[3] of a great number of animals, including primates.

With anti-human immune serum he obtained positive reactions with 100 per cent of the specimens of man and anthropoid apes examined, 92 per cent of the Old World monkeys (Cercopithecidæ), 78 per cent of the New World monkeys (Cebidæ), and 50 per cent of the marmosets (Hapalidæ). He found that the occurrence of positive reactions was directly proportional to the proximity to man as indicated by the usual criteria of classification.

Nuttall also made a smaller series of tests in which he estimated the volume of precipitate. With chimpanzee serum the volume was 130 per cent of that obtained with human serum, but the precipitate was less compact; with gorilla serum, 64 per cent; with orang serum, 42 per cent; with mandrill serum, 42 per cent; with baboon serum, 29 per cent; and with the serum of one of the New World monkeys, 29 per cent. Nuttall remarks that the figure for the orang is probably too low. Since, however, in precipitin reactions the amount of precipitate has a maximum at a certain concentration of the antigen, the estimation of this amount at an arbitrary dilution does not exactly measure the activity of a given serum. No statement was made by Nuttall as to the possibility of differentiating with certainty human and anthropoid serum by the precipitin reaction.

According to Chiò[4] anti-human serum reacts equally well on human and orang-utang serum. His results with anti-monkey serum were likewise in agreement with Nuttall's. Friedenthal[5] also asserts that the serological differentiation of man and the anthropoid apes cannot be made by any method. He had previously[6] tried to investigate the relationship of species by observing the occurrence or non-occurrence of hemoglobinuria after heterologous transfusions. The existence of individual differences alone makes this method unreliable. He noted that an injection of 25 cc. of human blood into a chimpanzee produced no symptoms. Friedenthal also reported that the blood of lower monkeys was hemolyzed *in vitro* by human serum and human blood by monkey serum.

Marshall[7] made a more careful study of human and monkey blood by means of normal and immune hemolytic sera. He found that normal heterologous sera (goat, sheep, ox, goose, and rabbit) were practically equally hemolytic for human and *Macacus* blood. Human anti-erythrocyte immune sera hemolyzed the bloods of *Macacus rhesus* and *Macacus cynomolgus* almost as well as human bloods, while

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[3] In the original publications the term blood is commonly used since in many cases solutions of blood were used for the tests. The tests, however, do not involve the blood cells but the serum protein.

[4] Chiò, M., *Atti r. Accad. sc.*, 1905–06, xli, 1093.

[5] Friedenthal, H., *Arch. Physiol.*, 1905, 1.

[6] Friedenthal, H., *Arch. Physiol.*, 1900, 494.

[7] Marshall, H. T., *J. Exp. Med.*, 1901–05, vi, 347.

anti-monkey serum was only feebly hemolytic for the human bloods examined. Differences between the two kinds of blood were also found by absorption experiments. Nevertheless, Marshall concluded that "there is quite a close relationship between the corpuscles of human beings and both of the varieties of monkeys examined."

There has been a considerable amount of work done[8–13] on the differentiation of human and monkey proteins, especially with regard to the forensic identification of blood stains. The results agree with those of Nuttall in showing that anti-human sera react with monkey serum, but less intensely than with human serum. The specificity of the reactions can be increased by previous saturation with monkey serum.[14,15]

One observation by von Dungern and Hirschfeld[16] may be considered as indicating a difference between human and chimpanzee blood. These authors tested the action of human Group III serum after absorption with human Group II blood, on the red cells of one chimpanzee. The agglutinins of three such sera were completely removed for chimpanzee and for human blood. But one serum thus treated would still agglutinate chimpanzee blood and none of several human bloods. It is impossible to decide with certainty from this observation whether the findings bespeak a constant species difference. (See the following paper of this series, pages 854 and 856.)

We know of no additional work on the serological properties of the blood of anthropoids since the paper of von Dungern and Hirschfeld (1911).

It is possible that the precipitin technique can be improved so as to yield additional information on the interrelationship of the primates. Another method which suggests itself is the use of anti-erythrocyte sera. In spite of the great amount of work on hemolysis and hemagglutination, these reactions have never been systematically exploited in the study of species relationship as has the precipitin reaction. The explanation for this neglect lies apparently in the presumption that the species specificities of the precipitins and of the hemagglutinins are of the same order and that species specificity means protein specific-

[8] Wassermann, A., and Schütze, A., *Berl. klin. Woch.*, 1901, xxxviii, 187.
[9] Uhlenhuth, *Deutsch. med. Woch.*, 1902, xxviii, 659, 679.
[10] Biondi, C., *Vierteljährschrift gerichtl. Med.*, 1902, xxiii, suppl. 1.
[11] Layton, E. N., *Tr. Chicago Path. Soc.*, 1903, v, 217.
[12] Ewing, J., *Proc. New York Path. Soc.*, 1903–04, iii, 14.
[13] Stern, R., *Deutsch. med. Woch.*, 1901, xxvii, 135.
[14] Fujiwara, K., *Deutsch. Z. ges. gerichtl. Med.*, 1922, i, 754.
[15] Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1924, xl, 91.
[16] von Dungern and Hirschfeld, *Z. Immunitätsforsch., Orig.*, 1910–11, viii, 526.

ity. It was tacitly assumed that the results in the two types of reaction would run parallel. Recently it has been pointed out (Landsteiner and van der Scheer[15,17,18]) that this supposition is unwarranted. As had been surmised before, the antigens which engender antibodies against red blood cells do not consist simply of proteins. It is possible to extract from erythrocytes by means of alcohol nonprotein substances, possibly of a lipoid nature, which have binding and immunizing properties. Certain inconsistencies regarding the antigens of red corpuscles have been reconciled.[17]

Considering the chemical dissimilarities of corresponding antigens, it is not surprising to find differences in the specificity of antibodies against the serum proteins and those against the red blood cells. One of the most striking and for our present purpose most important observations is this, that anti-erythrocyte immune bodies will regularly differentiate between species so closely related that their sera are indistinguishable by means of the precipitin reaction.[15,17] We have therefore undertaken to study the blood cells of the anthropoid apes by means of anti-erythrocyte sera.

EXPERIMENTAL.

Methods.—The two methods which we have mostly employed have been: first, the determination of the hemagglutinin titers of anti-erythrocyte sera on the blood cells of man and monkeys, and second, the determination of the fraction of agglutinin which remained after absorption by red blood cells of the various species. The titrations were made in the usual way in series of tubes containing decreasing concentrations (by halves) of inactivated immune serum. Each tube contained 0.5 cc. of the solution to which was added 1 drop of a 2.5 per cent suspension of washed blood cells. The readings were usually made after 1 or 2 hours at room temperature. For the absorptions, the immune sera, each suitably diluted according to its strength, were mixed with one-half volume of packed, washed red cells and allowed to stand at room temperature (up to 2 hours), during which time they were occasionally mixed by inversion of the tubes, and then kept in the refrigerator overnight. They were then centrifuged and the supernatant fluids titrated as described above.

The immune sera were prepared in the usual manner by a series of intravenous and intraperitoneal injections into rabbits of washed erythrocytes. Anti-human immune sera were prepared against blood cells of Groups I, II, and III.

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[17] Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1925, xli, 427; xlii, 123.

[18] Landsteiner, K., and van der Scheer, J., *J. Immunol.*, 1924, ix, 213.

Comparison of Human and Anthropoid Bloods.

Comparison of the titers of several anti-human immune sera for human and chimpanzee bloods respectively yielded no constant differences. Often the titers for chimpanzee blood were appreciably lower than for the human; but differences in the opposite direction

TABLE I.

Titration of Anti-Human Immune Sera with Human and Chimpanzee Erythrocytes.

	Group.	Cells.		
		Human.		Chimpanzee E.
		Group I.	Group II.	
		Highest effective dilution.		
Immune Serum 24.....	I	200	200	100
" " 26.....	I	500	250	375
" " 420.....	II	500	750	750
" " 421.....	II	3000	2000	2000

Reading after 2 hours at room temperature. Readings of all weak reactions were made microscopically in this as well as in the other experiments.

TABLE II.

Agglutination of Human and Chimpanzee Erythrocytes by Anti-Human Serum after Absorption with Chimpanzee Cells.

Anti-human erythrocyte serum 23 (Group I) (1/20 dilution) absorbed with cells of Chimpanzee F.

	Human blood cells.																						Chimpanzee E.	Chimpanzee F.
	33	34	35	36	37	38	39	40	18	25	28	8	W	5	6	7	8	9	10	11	12	13		
Group.	I	II	II	I	II	I	II	II	I	IV	I	III	II	I	I	I	II	IV	II	I	II	II		
	+±*	+	+±	+±	+	+	+	+	+	+	±	+	++	+	+	+±	+	+	+±	+	+	+	0	0

Tests made in small tubes with the following quantities.

1 drop absorbed immune serum.

1 drop salt solution.

1 drop 2.5 per cent suspension of washed blood cells.

Readings made after 2 hours at room temperature.

* In this and the following tables the signs have the following significance: 0 = negative; F. tr. = faint trace; Tr. = trace; ±, +, +, ±, ++, etc.

also occurred. The titers for human blood were also inconstant probably in part due to the difference in the blood group. The results of an experiment are shown in Table I. For other examples, see the absorption experiments below. Titrations with two anti-chimpanzee immune sera gave distinctly higher titers for the homologous bloods (see Table IV) in most cases.

In one experiment with orang blood the results were comparable to those with chimpanzee blood, while gibbon blood gave a definitely lower titer.

TABLE III.

Titration of Anti-Human Erythrocyte Serum after Absorption with Chimpanzee Cells.

Blood cells.	Anti-human erythrocyte serum 23 (Group I) (1/40 dilution).			Anti-human erythrocyte serum 344 (Group II) (1/20 dilution).		
	Control unabsorbed.	Absorbed with Chimpanzee E.	Absorbed with Chimpanzee F.	Control unabsorbed.	Absorbed with Chimpanzee E.	Absorbed with Chimpanzee F.
	Highest effective dilutions.					
Chimpanzee E.....	750	<40	<40	320	<20	20
" F.....	1000	320	<40	320	80	<20
" H.....	750	40	<40	320	<20	<20
" I.....	500	<80	<40	320	<20	<20
Human 72 (Group I).....	4000	2560	640	1600	1280	320
" 77 (" II).....	2000	640	160	800	320	320
" 78 (" III).....	2000	1280	320	800	640	240
" L (" IV).....	2000	640	320	800	320	240

Reading after 1 hour at room temperature.

Absorption Experiments.—Experiments in which anti-human immune sera were absorbed with chimpanzee erythrocytes and *vice versa* yielded unequivocal and constant results. Such human bloods must be selected which will not leave behind any group-specific agglutinins. After removal of the agglutinins for chimpanzee blood, such liquids always contained agglutinins for human bloods of all groups. We have tested a great number (68) of human bloods in this manner since it became evident that considerable individual variation exists. The bloods of four chimpanzees were used. Table II is given as an example.

To determine the proportion of agglutinins in anti-human immune

serum which act on both human and chimpanzee bloods, and those which are specific for human blood alone, the supernatants of the absorbed sera were titrated. In consideration of the existence of individual differences in human bloods, the fluids were tested on a great number of blood specimens, especially selected for their dissimilarities. Table III shows the findings in a typical absorption experiment.

The tests show that in all cases with appropriate sera a considerable portion of the agglutinating capacity of the serum remained after absorption with the heterologous blood. This portion approximated in

TABLE IV.

Titration of Anti-Chimpanzee Immune Serum after Absorption with Human Blood Cells.

Serum 475 (1/10 dilution) absorbed with human erythrocytes (Group I).			Serum 474 (1/20 dilution) absorbed with human erythrocytes (Group II).		
Blood cells.	Control unabsorbed.	Absorbed.	Blood cells.	Control unabsorbed.	Absorbed.
Human (Group I).....	120	<10	Human (Group I).....	200	<40
" (" I).....	240	<10	" (" II).....	200	<20
" (" I).....	80	<10	" (" III).....	400	20
" (" I).....	120	<10	Chimpanzee E.....	400	120
" (" II).....	80	<10	" F.....	600	160
" (" III).....	60	<10			
Chimpanzee E.....	320	160			

Reading after 1 hour at room temperature.

the case of immune serum 344, 20 to 40 per cent after absorption with blood of Chimpanzee F; 40 to 80 per cent after absorption with blood of Chimpanzee E (see Table V). The fraction acting on both bloods was therefore between 20 and 80 per cent. It will be observed in Table III that individual differences were not limited to human bloods, for the blood of one chimpanzee showed a peculiarity. After absorption with the cells of Chimpanzee E as a rule the immune serum still contained an appreciable quantity of agglutinins for Chimpanzee F. This observation held true for this blood in a number of other tests. Absorption with the cells of Chimpanzee F, on the other hand, removed all of the agglutinins for all of the chimpanzee bloods tested, and in all instances left behind agglutinins for human blood. Analo-

gous experiments were made with several different immune sera. It is quite possible that similar results could be obtained with the serum of normal animals, but we have not made such experiments. Results confirming those reported were obtained when anti-chimpanzee immune sera were absorbed with human blood (see Table IV).

The conclusion to be drawn from these experiments is that it is regularly possible to differentiate human and chimpanzee bloods by means of suitable anti-erythrocyte immune sera.

As we had at our disposal only small quantities of orang blood only one absorption experiment was made on anti-human serum with orang blood. The absorbed liquid was insufficient for titrations, and the tests were made with single drops as in the experiments in Table II. The experiment demonstrated that orang blood differs from human blood and indicated that the relationship between orang and chimpanzee bloods is closer than it is between orang and human. Agglutinins remained for the eight human bloods tested and to a larger degree for that of Chimpanzee F, which was shown before to have an individual peculiarity, while all the agglutinins for the other two chimpanzees and four orangs were absorbed.

It has been claimed by Bruck[19] that the sera of various human races can be differentiated by the complement fixation reaction; but this has been denied by other authors.[20,21] We have examined by the methods employed in the other experiments the blood of negroes. Our experiments show that if serological differences do exist between the bloods of white men and American negroes—no longer a pure race—they are much smaller than those between man and the anthropoid apes. So far we have been unable to demonstrate any characteristic difference. It is not impossible, however, that slight differences might be found if individuals of several races preferably of pure blood were carefully studied by this method in all of its modifications.

Comparison of the Bloods of Man and the Anthropoid Apes with Those of the Lower Monkeys.

In another series of experiments we have studied the serological relationship of the erythrocytes of man and chimpanzee to those

[19] Bruck, C., *Berl. klin. Woch.*, 1907, xliv, 793.

[20] Marshall, H. T., and Teague, O., *Philippine J. Sc., Sect. B*, 1908, iii, 357.

[21] Fitzgerald, J. G., *J. Med. Research*, 1909, xxi, 41.

of the lower monkeys. Table V shows that immunization with the bloods of lower monkeys (baboon and macaque) did not engender any considerable quantity of antibodies against the blood of the higher apes, and *vice versa*. It should be noted that the sera employed in the absorption did not have a very high titer and that some previous experiments showed there was a somewhat greater fraction of agglutinins acting on both bloods in question (see references 7 and 18).

TABLE V.

Titration of Various Anti-Erythrocyte Immune Sera after Absorption with Various Primate Blood Cells.

Bloods.	Anti-human erythrocyte serum 344 (Group II) (1/20 dilution).			Anti-chimpanzee erythrocyte serum 474 (1/20 dilution).			Anti-baboon erythrocyte serum 458 (1/20 dilution).			Anti-macaque erythrocyte serum 456 (1/20 dilution).		
	Control unabsorbed.	Absorbed by baboon cells.	Absorbed by macaque cells.	Control unabsorbed.	Absorbed by baboon cells.	Absorbed by macaque cells.	Control unabsorbed.	Absorbed by human Group I cells.	Absorbed by chimpanzee cells.	Control unabsorbed.	Absorbed by human Group II cells.	Absorbed by chimpanzee cells.
Human (Group II).....	400	400	400	160	160	160	80	<20*	20	60	<20	40
Chimpanzee.....	200	200		640	640	480	80	80	<20	40	20	<20
Baboon.....	80	<20	<20	40	20	<20	600	400	400	800	800	800
Macaque.....	80	40	<20	40	<20	<20	400	300	200	800	800	800

Reading after 1 hour at room temperature.
*Test made with Group I blood.

When absorbed with monkey blood the agglutinating powers of both the anti-human and anti-chimpanzee sera were not markedly affected. A similar result was obtained on treating anti-baboon and anti-macaque sera with human and chimpanzee blood respectively. Also in this experiment a distinct difference between human and chimpanzee blood could not be demonstrated.

Comparison of the Bloods of the Lower Monkeys.

With the method employed above a difference was demonstrable between the two genera of Old World monkeys (Catarrhina) investi-

TABLE VI.

Titration of Anti-Baboon Erythrocyte Serum after Absorption with Macaque Blood Cells.

Serum 458 (1/20 dilution) absorbed with erythrocytes of *Macacus rhesus*.

Bloods.	Control unabsorbed.	Absorbed.
Human Group II	80	80
Chimpanzee F	80	80
Baboon	1000	320
Macaque	500	<20

Reading after 1 hour at room temperature.

TABLE VII.

Titration of Anti-Macaque Erythrocyte Serum after Absorption with Baboon Blood Cells.

Serum 456 (1/20 dilution) absorbed with erythrocytes of baboon.

Bloods.	Control unabsorbed.	Absorbed.
Human (Group I)	40	20
Chimpanzee E	40	20
Baboon	1600	<20
Macaque	800	40

Reading after 1 hour at room temperature.

TABLE VIII.

Titration of Anti-Baboon and Anti-Macaque Immune Sera after Absorption with Sapajou Erythrocytes.

Blood cells.	Anti-baboon immune serum 458 (1/20 dilution). Absorbed with blood cells of sapajou (*Cebus hypoleucus*).		Anti-macaque immune serum 456 (1/20 dilution). Absorbed with blood cells of sapajou (*Cebus hypoleucus*).	
	Control unabsorbed.	Absorbed.	Control unabsorbed.	Absorbed.
Baboon	640	640	640	640
Macaque	640	640	320	320
Sapajou	80	<20	80	<20

Reading after 3 hours at room temperature.

gated, more especially by the use of the antiserum against the baboon in comparison with that against the macaque. This may have been due to an accidental circumstance (see Tables VI and VII).

The differences between the two species of Catarrhina and of one New World monkey—ringtail or sapajou monkey (*Cebus hypoleucus*)—were very marked. In this case the titration alone showed a striking specificity.

The absorption of the anti-baboon and anti-macaque sera with the blood of the sapajou did not diminish the titer for the cells of baboon and macaque (Catarrhina).

DISCUSSION.

The experiments described show that a definite and constant serological difference is demonstrable between the bloods of man and the two anthropoids studied—chimpanzee and orang-utang. The experiments also show that this method of agglutination by absorbed immune sera is suitable for the demonstration of serological differences between the bloods of species so closely related that they are indistinguishable by the precipitin reaction as it is usually employed. The difference between the bloods of the lower monkeys on the one hand and of man and the anthropoids on the other is considerably greater than that between the two latter, as is seen from the titers of the immune sera and the results of absorption experiments. The experiments permit of a further consideration. Both anti-human and anti-chimpanzee serum, after absorption with the blood of a lower monkey, do not lose an appreciable amount of their agglutinin content. Also anti-monkey sera behave quite similarly when treated either with chimpanzee or with human blood. It may be concluded, therefore, that anthropoid blood is not much more similar to that of the lower monkeys than is the blood of man to that of lower monkeys. This conclusion is in agreement with the accepted view of zoologists that man has not evolved directly from any of the existing species of primates, as was formerly supposed, but that the Catarrhina, anthropoids, and man have all sprung from a common stock. (See the diagrammatic representation in the third paper of this series, page 871.)

It is of interest to compare the degree of difference which we have

found between the bloods of man and chimpanzee with that already observed between the bloods of other pairs of closely related animals. In the case of the horse and donkey,[18] for instance, the proportion of homologous agglutinins left after absorption with the heterologous blood is the same or greater than in the case of man and chimpanzee, when one makes allowance for individual differences. Measured by the agglutinin reaction bloods of the latter pair would seem to be no farther distant than those of the horse and donkey, between which hybridization is easily possible. It must not be overlooked, however, that horse and donkey are regarded as considerably closer related morphologically than are man and apes.

SUMMARY.

While the precipitin tests do not differentiate, according to the results of previous workers, between the serum proteins of man and chimpanzee, a clear-cut differentiation between the blood cells of man and the anthropoids was obtained by means of hemagglutinins.

According to our tests on bloods of whites and negroes, constant racial serological differences among human bloods, if they exist at all, are certainly smaller than the differences between the bloods of man and the anthropoid apes.

The serological differences between man and the lower monkeys appear to be no greater than those between the anthropoid apes and the lower monkeys. These findings confirm the opinion that the anthropoid apes do not rank in the genealogical tree between lower monkeys and man.

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