ON THE SPECIFICITY OF AGGLUTININS AND PRECIPITINS.*

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The knowledge of serological reactions has opened a field of research dealing with the specific configurations existing in the chemical constituents of organisms. The results of agglutinin and lysin reactions and the use of the absorption method have disclosed an unexpected variety of structures as exemplified by the existence of differently reacting groups in a single kind of cells, of differences between individuals of the same species, of various types among bacilli of the same sort, and so on.

The question whether the methods of absorption or partial saturation applied to precipitin reactions will disclose analogous facts is discussed in the present paper. Should this be the case, it might be possible to isolate antibodies which could serve to discriminate chemical groups contained in the protein molecules involved in precipitin reactions. For present purposes the nature of the precipitins reacting strongly on antigens of remote species will be left out of account because in their case special factors are possibly concerned. These reactions have recently been studied by Friedberger and his coworkers[1] and have been termed by them "heterogenetic precipitations."

Experiments on Partial Saturation of Precipitins.

The experiments on partial saturation of precipitins reported by previous writers include instances of two sorts. In the one an

* Nineteenth paper on antigens and specificity.

[1] Friedberger, E., and coworkers, *Z. Immunitätsforsch., Orig.*, 1919, xxviii, 237; 1920, xxx, 351; 1923, xxxvi, 233, 272, 386; 1923–24, xxxviii, 184, 264; *Biochem. Z.*, 1923, cxxxvii, 312; *Klin. Woch.*, 1922, i, 1248. *Cf.* Manteufel, P., and Beger, H., *Z. Immunitätsforsch., Orig.*, 1921–22, xxxiii, 348. Yu, I., *Centr. Bakt., 1. Abt., Orig.*, 1923, xc, 381. Wells, H. G., Meeting of the American Association of Immunologists, Boston, 1923.

antiserum is obtained through the injection of an antigen composed of several serologically different proteins. The several antibodies which it in consequence contains are thrown down separately by the consecutive addition of the individual antigenic components. Experiments of this kind have been performed by Ascoli[2] Ide,[3] von Dungern,[3] and others with the proteins of serum—globulin, albumin—which, as it happens, behave serologically like entirely different substances.[4] In instances of the second sort, a precipitin is produced by the injection of a single protein or of a mixture of proteins of the same animal derivation. These precipitins react, though to a lesser degree, upon the proteins of related species. In such cases it is possible to assume that the precipitation occurring with material from related species comes about as a result of a similarity in the chemical composition of the strange material and that used as antigen. Several authors have investigated the problem thus raised by means of experiments involving the partial saturation of precipitins.[5] The conclusion is generally drawn, in conformity with the experiments of Ehrlich and Morgenroth,[6] that the reactions of a single immune serum with several antigens are referable to a multiplicity of antibodies corresponding to various chemical groups in the homologous antigen, some of these groups being present also in the related antigens and thus giving rise to the overlapping reactions.

The method of partial saturation of precipitins has been but little used for biological and even practical purposes.[7] While this is possibly due to the technical difficulty of determining the proper conditions of

[2] Ascoli, M., *Münch. med. Woch.*, 1902, xlix, 1409.

[3] Ide, M., *Bull. Acad. Roy. mêd. Belg.*, 1903, xvii, series 4, 913. von Dungern, E. F., *Centr. Bakt., 1. Abt., Orig.*, 1903, xxxiv, 355; Die Antikörper, Jena, 1903.

[4] Dale, H. H., and Hartley, P., *Biochem. J.*, 1916, x, 408. Doerr, R., and Berger, W., *Z. Hyg. u. Infectionskrankh.*, 1922, xcvi, 191.

[5] Weichardt, W., *Hyg. Rundschau*, 1903, xiii,756; *Z. Med.-Beamte*, 1902, xv, 729. Ide.[3] von Dungern.[3] Manteufel, T., and Beger, H., *Z. Immunitätsforsch., Orig.*, 1921–22, xxxiii, 367, 370. Welsh, D. A., and Chapman, H. G., *J. Hyg.*, 1910, x, 177. Ascoli.[2]

[6] Ehrlich, P., and Morgenroth, J., *Berl. klin. Woch.*, 1901, xxxviii, 569, 598.

[7] *Cf.* Uhlenhuth, P., *Beihefte med. Klin.*, 1907, iii, 246, 252.

the experiments,[8] the principle of the method is likewise open to doubt. For after removing the precipitate formed by the addition of an heterologous antigen, a quantity of this latter may still be present in the fluid, side by side with, or perhaps in a soluble combination with, residual antibodies.[9] It is conceivable that such a mixture may not react upon a further addition of heterologous antigen and yet will do so with an homologous antigen endowed with higher affinity. As a matter of fact, the supernatant fluid remaining after partial precipitation with an homologous antigen reacts with this same substance, if it be added in a higher concentration than that used for the first precipitation, as Coca and Kosakai[10] have shown by experiments with the pseudoglobulin of serum and crystalline egg albumin. From similar experiments which we ourselves have made, it will be seen that the solutions, after partial neutralization, did not only behave like higher dilutions of antiserum, but actually showed a relatively rapid decrease in the intensity of the reaction when the concentration of antigen was diminished (Table 1).

3 cc. of horse serum, diluted 1:100, were mixed with 0.6 cc. of rabbit anti-horse precipitin, kept for 2 hours at room temperature and overnight in the ice box, and then centrifuged. 0.5 cc. of the supernatant fluid was taken for every test and 0.1 cc. of various dilutions of horse serum added. In the same way dilutions of untreated immune serum were tested. Readings were made after 3 hours standing at room temperature and overnight in the refrigerator. The strength of the reactions is indicated as follows: F. tr. = faint trace; Tr. = trace; ±, +, +±, etc.

All the immune sera employed were obtained from rabbits immunized in the usual way by weekly intraperitoneal or intravenous injections.

Like results were obtained in similar tests with an anti-human precipitin.

The following experiments show the effects of partial saturation with, and subsequent addition of, homologous and heterologous antigen.

[8] Fürth, J., *Arch. Hyg.*, 1923, xcii, 158.

[9] *Cf.* Zinsser, H., and Young, S. W., *J. Exp. Med.*, 1913, xvii, 396. Michaelis, L., and Fleischmann, P., *Z. exp. Path. u. Therap.*, 1905, i, 547. Opie, E. L., *J. Immunol.*, 1923, viii, 19. Nachtergael, A., *La Cellule*, 1905, xxii, 125.

[10] Coca, A. F., and Kosakai, M., *J. Immunol.*, 1920, v, 297.

To a series of tubes containing 1.5 cc. of various dilutions of human and monkey serum, 0.3 cc. anti-human precipitin was added. After 2 hours at room temperature and overnight in the ice box, the tubes were well centrifuged in order to remove the precipitates formed. After centrifugation some of the tubes still showed slight turbidity, which was allowed for in the readings.

To 0.4 cc. of the supernatant fluid, 0.2 cc. of a 1:500 dilution of human and monkey serum (*Macacus rhesus*) was added (Table II, *a*). In another series of tubes, there was added instead the antigen used for the previous precipitation in a concentration five times greater than before (Table II, *b*). Readings were made after 2 hours standing at room temperature.

TABLE I.

Horse serum diluted.	Supernatant fluid.	Untreated immune serum diluted.				
		1:6	1:10	1:20	1:50	1:100
1:50	+	+++	+++	+±	±	Tr.
1:100	±	++	+++	++	±	F. tr.
1:250	Tr.	+±	++	++±	±	" "
1:500	0	+	+±	+±	±	" "
1:1,000	0	+	+	+	±	" "

TABLE II, *a*.

Supernatant fluid after precipitation with human serum diluted.	Human serum 1:500.	Monkey serum 1:500.	Supernatant fluid after precipitation with monkey serum diluted.	Human serum 1:500.	Monkey serum 1:500.
1:1,000	+±	+±	1:1,000	+±	Tr.
1:300	+	Tr.	1:300	+±	"
1:100	Tr.	0	1:100	+±	F. tr.
1:30	0	0	1:30	+	0
1:15	0	0	1:15	+	0

It will be seen from the experiment that anti-human precipitin saturated with monkey serum reacts specifically on human protein. Evidently, therefore, the serological differentiation between monkey and human serum is not particularly difficult, as Fujiwara has recently shown anew.[11] However, by using higher concentrations of the antigen, the fact is demonstrable that the liquid resulting from the partial saturation still contains antibodies reacting on monkey serum.

[11] Fujiwara, K., *Z. ges. gerichtl. Med.*, 1922, i, 754.

A similar experiment was made with anti-chicken precipitin, chicken, and goose serum, the quantitative relations being the same (Table III, *a*). Additional tests in which various concentrations of homologous antigen were added to the supernatant fluid are summarized in Table III, *b*. Readings were made after 4 hours at room temperature (*cf.* Table IX).

TABLE II, *b*.

Supernatant fluid after precipitation with human serum diluted.	Human serum.*	Supernatant fluid after precipitation with monkey serum diluted.	Monkey serum. *
1:1,000	++	1:1,000	Tr.
1:300	+	1:300	+
1:100	+	1:100	+
1:30	+±	1:30	+±
1:15	Tr.	1:15	Tr.

* Concentration five times greater than that indicated in Column 1.

TABLE II, *c*.

0.4 cc. untreated immune serum diluted.	0.2 cc. human serum 1:500.
1:6	++
1:12	+±
1:24	+
1:48	Tr.
1:96	F. tr.

TABLE II, *d*.

	1.5 cc. human serum diluted.					1.5 cc. monkey serum diluted.				
	1:15	1:30	1:100	1:300	1:1,000	1:15	1:30	1:100	1:300	1:1,000
0.3 cc. immune serum	++	++	+++	++	+	++	+±	+	+±	+

In order to avoid, to a certain extent, the complications arising from the presence of several proteins in serum (*cf.* the results by Opie[9]) an experiment similar to the foregoing one was made with a precipitin for horse euglobulin. For the immunization, the fraction precipitated at a 33 per cent saturation with ammonium sulfate was used. The immune serum proved to be highly specific when solutions of euglobulin and albumin—precipitated between 55 and 100

per cent saturation with ammonium sulfate—were tested. With the latter substance no reaction took place up to concentrations of 1 to 2 per cent.

TABLE III, *a*.

Supernatant fluid after precipitation with chicken serum diluted.	Chicken serum 1:500.	Goose serum 1:500.	Supernatant fluid after precipitation with goose serum diluted.	Chicken serum 1:500.	Goose serum 1:500.
1:1,000	+±	+±	1:1,000	+±	+
1:300	+	±	1:300	+	0
1:100	F. tr.	0	1:100	±	0
1:30	0	0	1:30	Tr.	0
1:15	0	0	1:15	"	0

TABLE III, *b*.

Supernatant fluid after precipitation with chicken serum diluted.	Chicken serum.					Supernatant fluid after precipitation with goose serum diluted.	Goose serum.				
	1:200	1:60	1:20	1:6	1:3		1:200	1:60	1:20	1:6	1:3
1:1,000	++	+++	++	+	+±	1:1,000	+	+	+	++	+±
1:300	+±	+	+	+	+±	1:300	Tr.	±	+	+±	±
1:100	Tr.	±	+	+	+±	1:100	0	Tr.	+	+	±
1:30	0	Tr.	±	±	±	1:30	0	0	Tr.	0	Tr.
1:15	0	0	0	0	0	1:15	0	0	0	0	0

TABLE III, *c*.

0.4 cc. untreated immune serum diluted.	0.2 cc. chicken serum 1:500.	0.2 cc. goose serum 1:500.
1:6	++	++
1:12	+±	+
1:24	+	±
1:48	±	F. tr.
1:96	Tr.	0
1:192	0	0

The results (Table IV) are like those obtained with precipitins against whole serum. However, in other experiments with two sera containing antibodies effective against horse serum albumin, the phenomenon of apparent specificity after partial neutralization with donkey serum was hardly perceptible.

A peculiarity noted in the experiment with horse and chicken immune serum (Tables III and IV) is the decrease in intensity of the specific reaction when the amount of heterologous antigen taken for the partial saturation is increased.

When antigens unrelated serologically are tested, the results are different (Table V).

In such an experiment the strength of the reaction was not influenced by the quantity of heterologous antigen employed in the

TABLE IV, *a*.

Readings made after 2 hours.

Supernatant fluid after precipitation with horse serum diluted.	Horse serum 1:500.	Donkey serum 1:500.	Supernatant fluid after precipitation with donkey serum diluted.	Horse serum 1:500.	Donkey serum 1:500.
1:1,000	+	±	1:1,000	+	Tr.
1:300	Tr.	F. tr.	1:300	+	F. tr.
1:100	0	0	1:100	±	0
1:30	0	0	1:30	0	0
1:15	0	0	1:15	0	0

TABLE IV, *b*.

Supernatant fluid after precipitation with horse serum diluted.	Horse serum.*	Supernatant fluid after precipitation with donkey serum diluted.	Donkey serum.*
1:1,000	+±	1:1,000	+
1:300	++	1:300	+
1:100	+	1:100	+
1:30	±	1:30	±
1:15	Tr.	1:15	Tr.

* Concentration five times greater than that indicated in Column 1.

initial precipitation. In cases in which a decrease is observed, one may ascribe it either to a reaction of the heterologous antigen with almost the whole amount of antibodies in forming a precipitate or to inhibition of the precipitation by an excess of antigen.[12] This latter phenomenon, which is probably due to

[12] Camus, M. L., *Compt. rend. Acad.*, 1901, cxxxii, 215. Halban, J., and Landsteiner, K., *Münch. med. Woch.*, 1902, xlix, 473. Michaelis, L., *Beitr. chem. Physiol. u. Path.*, 1904, iv, 59. Eisenberg, F., *Bull. internat. Acad. sc. Cracovie*, 1902, 289.

the formation of a soluble compound between antibody and antigen, is also indicative of a specific affinity. The phenomenon of inhibition is not equally well pronounced with every precipitin serum. But if the serum is appropriate, even those related heterologous antigens which give only a weak precipitation as compared with the homologous antigen have the power to inhibit the reaction with the homologous antigen to a high degree. An example is furnished by tests with a precipitin for rat serum which gave also a weak reaction with mouse serum (Table VI). A number of normal sera were employed for comparison.

TABLE V.

The arrangement of the experiment was like that of the previous ones. Immune serum against whole horse serum was employed, and for the neutralization and the tests, solutions of euglobulin and albumin of horse serum. The undiluted solution contained about 7.5 per cent protein. Readings were made after 2 hours at room temperature and overnight in the ice box.

Supernatant fluid after precipitation with albumin solution diluted.	Euglobulin solution 1:500.	Albumin solution 1:500.
1:1,000	++	0
1:300	++	0
1:100	++	0
1:30	++	0
1:15	++	0
Untreated immune serum.		
1:6	++	++

It would appear from Table VI that despite the wide difference in strength exhibited in the direct reaction, mouse serum inhibits almost as well as rat serum. The weaker but not insignificant inhibition produced by some sera of Ungulata may be ascribed, perhaps properly, to a certain affinity of the antibodies for these proteins and in that respect it is worth noting that no alteration at all was brought about by the sera of birds.

We have tried to effect the absorption of precipitins by a method similar to that used for agglutinins[13] and have employed for this purpose proteins coagulated by heat or alcohol. The results were not wholly satisfactory owing to the difficulty of obtaining clear solutions after centrifugation, as also because of the

[13] *Cf.* Friedberger, E., and Meissner, G., *Z. Immunitätsforsch., Orig.*, 1923, xxxvi, 233

large amounts of coagulated material necessary for complete absorption, and because small amounts of the proteins went into solution, as shown by addition of precipitin. The outcome of the tests was similar to that obtained by the method of partial precipitation with soluble proteins. The amount of apparently specific antibodies left behind after a treatment with coagulated heterologous protein seemed to be considerably smaller than in the analogous tests with hemagglutinins, material from the same species being employed in both cases (see Table VIII). In judging the results of such experiments, the fact has to be taken into account that a decrease in the quantity of antibodies often affects the strength of the heterologous reaction more than that of the homologous one.

TABLE VI, *a*.

0.1 cc. of precipitin for rat serum. 0.2 cc. of dilutions of rat, mouse, and guinea pig serum. Readings made after 1 hour at room temperature.

Rat serum diluted.						Mouse serum diluted.						Guinea pig serum diluted.					
1:20	1:50	1:150	1:500	1:1,000	1:2,000	1:20	1:50	1:150	1:500	1:1,000	1:2,000	1:20	1:50	1:150	1:500	1:1,000	1:2,000
+++	++++	+++	++±	+	Tr.	±	+	±	±	Tr.	F. tr.	0	0	0	0	0	0

TABLE VI, *b*.

To 0.1 cc. of precipitin for rat serum were added 0.2 cc. of various normal sera (to the control tube 0.2 cc. of saline) and 10 minutes later 0.1 cc. of rat serum diluted 1:125. Readings made after 1 hour.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Control.
+±	+±	+±	++	F. tr.	Tr.	++	+	+±	+	++	+	+	+±	++	++	++	++

1, human; 2, monkey; 3, cat; 4, rabbit; 5, rat; 6, mouse; 7, guinea pig; 8, pig; 9, goat; 10, sheep; 11, beef; 12, horse; 13, donkey; 14, mule; 15, chicken; 16, goose; 17, pigeon.

Partial Saturation of Precipitins against Azoproteins.—As a further means of investigating the problem under discussion, we have made use of precipitins for antigens containing specific groups of known chemical constitution.[14] Sera were prepared by injecting rabbits with an azo compound formed by coupling horse serum with diazotized meta-amino-benzene-sulfonic (metanilic) acid. For *in vitro* tests

[14] Landsteiner, K., and Lampl, H., *Z. Immunitätsforsch., Orig.*, 1917, xxvi, 258, 293; *Biochem. Z.*, 1918, lxxxvi, 343; 1919, xciii, 106; 1920, civ, 280.

azo compounds prepared from chicken serum were employed.[14] As established previously, such sera react strongly with the homologous (metanilic acid) compound but also to a lesser degree on some other azoproteins with chemically similar azo components. For the heterologous reaction, the azo compound formed by coupling chicken serum with *p*-chlor-*o*-amino-benzene-sulfonic acid C_6H_3 $(NH_2)^1$ $(SO_3H)^2$ Cl^4.

TABLE VII, *a*.

Technique as already given; readings made after 4 hours at room temperature; azoprotein from metanilic acid = M, from *p*-chlor-*o*-amino-benzene-sulfonic acid = C. The solution of azoprotein contained about 5 per cent protein.

Supernatant fluid after precipitation with M diluted.	Solution M 1:500.	Solution C 1:500.	Supernatant fluid after precipitation with C diluted.	Solution M 1:500.	Solution C 1:500.
1:1,000	0	0	1:1,000	+	Tr.
1:300	0	0	1:300	+	0
1:100	0	0	1:100	±	0
1:30	0	0	1:30	±	0
1:15	0	0	1:15	Tr.	0

TABLE VII, *b*.

To 0.2 cc. of various dilutions of Solutions M and C was added 0.04 cc. of precipitin for M. Readings were made after 2 hours at room temperature.

Solution M diluted.						Solution C diluted.					
1:15	1:30	1:100	1:300	1:1,000	1:3,000	1:15	1:30	1:100	1:300	1:1,000	1:3,000
±	±	+±	++	++	+	0	Tr.	+	+	+	±

was taken. As shown in Table VII, the immune serum gave a precipitation with this substance distinctly weaker than that with the homologous antigen, in analogy with the group reactions of natural antigens.

Since the chemical constitution of the specific groups of the two substances mentioned is fully known, a valid conclusion can be drawn. From a chemical point of view it is impossible to believe that two kinds of antibodies developed in the serum of the immunized animal, one of them acting on a common group of both antigens, the other on a

special group of the homologous material. On the other hand, a reaction of one antibody on the two chemically similar groups is quite conceivable, and the conception is supported by previous work.[14] The fact remains that after partial saturation with the homologous substance, the same occurrence took place as in the similar experiments with natural antigens; namely, a specific reaction of the supernatant fluid with the homologous, more active antigen. Similar results could undoubtedly be obtained with other azoproteins.

Experiments on Hemagglutinins.

The following tests were performed in order to compare the specificity and partial absorption of hemagglutinins and precipitins. Experiments of the sort with immune agglutinins and lysins have been carried out by several authors,[15] following the well known work of Ehrlich and Morgenroth.

The titration was made by mixing 0.5 cc. of various dilutions of rabbit immune agglutinins inactivated in the usual way with 0.05 of a 2.5 per cent emulsion of washed blood (0.1 cc. in the case of the emulsion of bird blood). The readings were made after 2 hours standing at room temperature. The figures given in Table VIII indicate the highest dilutions at which agglutination could still be discerned microscopically.

For absorption one-half volume of well centrifuged blood sediment from various species of animals was added to the immune serum diluted 1:10 to 1:40. The mixture was kept for 2 hours at room temperature and overnight in the ice box. Hemolytic tests were made only occasionally. They seemed to parallel the results of the agglutination tests.

It is evident from Table VIII that the titer of the hemagglutinins contained in the immune rabbit serum may be quite different for related species, as is shown, for instance, in the tests with human and monkey red cells. The lack of parallelism between these findings and those with precipitins is seen still more strikingly in the tests with anti-chicken immune serum.

[15] von Dungern, E., and Hirschfeld, L., *Z. Immunitätsforsch., Orig.*, 1909–10, iv, 531. Landsteiner, K., and Reich, M., *Z. Hyg. u. Infectionskrankh.*, 1907–08, lviii, 213. Morgenroth, J., and Bieling, R., *Biochem, Z.*, 1922, cxxxi, 525.

The high titer of the chicken agglutinins for goose blood is probably referable to the normal rather than to immune agglutinins since similar titers have been noted with normal rabbit serum. Moreover, these agglutinins were not absorbed by chicken blood.

TABLE VIII.

	Blood.	
	Human.	Monkey.
Agglutinin for human blood I	1:3,000	1:240
" " " " I 1/20 absorbed with human blood	1:80	1:40
Agglutinin for human blood I 1/20 absorbed with monkey blood	1:2,000	1:<20
Agglutinin for human blood II	1:4,000	1:640
" " " " II 1/20 absorbed with monkey blood	1:3,000	1:<20
Agglutinin for human blood III	1:1,000	1:100

	Guinea pig.	Mouse.	Rat.
Agglutinin for guinea pig blood I	1:1,000	1:160*	1:60*
" " " " " II	1:1,000	1:160*	1:80*
" " rat blood		1:3,200	1:6,400
" " " " 1/20 absorbed with rat blood		1:20	1:20
Agglutinin for rat blood 1/20 absorbed with mouse blood		1:20	1:3,200

	Horse.	Donkey.
Agglutinin for horse blood I	1:10,240	1:2,560
" " " " I 1/10 absorbed with horse blood	1:10	1:10
Agglutinin for horse blood I 1/10 absorbed with donkey blood	1:2,560	1:20
Agglutinin for donkey blood I	1:1,600	1:3,200
" " " " I 1/10 absorbed with horse blood	1:10	1:800
Agglutinin for donkey blood I 1/10 absorbed with donkey blood	1:160	1:<10
Agglutinin for donkey blood II	1:8,000	1:12,000
" " " " II 1/40 absorbed with horse blood	1:40	1:6,400
Agglutinin for donkey blood II 1/40 absorbed with donkey blood	1:60	1:40

* Similar titers were found with normal rabbit serum.

TABLE VIII—*Concluded.*

	Blood.			
	Chicken.	Guinea hen.	Goose.	Pigeon.
Agglutinin for chicken blood I	1:800	1:300	1:1,600*	1:40
" " " " I 1/10 absorbed with chicken blood	1:<20	1:80	1:1,600	1:40
Agglutinin for chicken blood I 1/10 absorbed with guinea hen blood	1:640	1:<20	1:1,600	1:40
Agglutinin for chicken blood I 1/10 absorbed with goose blood	1:800	1:200	1:40	1:40
Agglutinin for chicken blood I 1/10 absorbed with pigeon blood	1:800	1:400	1:1,600	1:<20
Agglutinin for chicken blood II	1:1,200	1:200	1:400*	1:100
" " " " II 1/10 absorbed with chicken blood	1:<10	1:20	1:400	1:10
Agglutinin for chicken blood II 1/10 absorbed with guinea hen blood	1:800	1:<10	1:400	1:10
Agglutinin for chicken blood II 1/10 absorbed with goose blood	1:1,600	1:200	1:10	1:160
Agglutinin for chicken blood III	1:800	1:400	1:800*	1:80
" " " " III 1/10 absorbed with chicken blood	1:<10	1:20	1:800	1:10
Agglutinin for chicken blood III 1/10 absorbed with guinea hen blood	1:800	1:<10	1:800	1:10
Agglutinin for chicken blood III 1/10 absorbed with goose blood	1:1,600	1:400	1:20	1:120

For comparison with the agglutinin tests, an experiment on the specificity of chicken precipitin is presented in Table IX.

The low degree of specificity instanced by this case has been observed before by Uhlenhuth[16] among others, and Higashi[17] has had similar findings in the precipitation of bird hemoglobin. As a result of such observations, the idea has been put forward that species specificity as shown by serological tests is less pronounced in birds than in mammals. But we incline to think the phenomenon due to the wide distance of the bird from the animal used for obtaining the immune serum; that is to say, the rabbit. In keeping with this idea is the higher degree of specificity of the precipitin reactions obtained with immune sera derived from rabbits, within the order of Rodentia, as already established by previous work[18] (see Table VI, *a*).

[16] Uhlenhuth, P., *Deutsch. med. Woch.*, 1905, xxxi, 1673.

[17] Higashi, S., *J. Biochem.*, 1923, ii, 315.

[18] Trommsdorff, R., *Arb. k. Gsndhtsamte*, 1909, xxxii, 560. Graetz, F., *Z. Immunitätsforsch., Orig.*, 1910, vi, 627; *cf.* Steffenhagen, K., and Schoenburg, *Z. Immunitatsforsch., Orig.*, 1910–11, viii, 563.

In all cases, after treatment of the immune serum with heterologous blood, a fraction was obtained which was highly active and specific for the homologous blood, even when such closely related species as the horse and donkey were tested. In this case the original titer may have been nearly or quite the same for both kinds of blood. The result was not essentially different after a second similar treatment with the heterologous blood. These findings indicate that the differentiation of blood of closely related species is often easier by means of agglutinins than by precipitin reactions. Agglutinins can very probably be employed when precipitation tests meet with difficulty, for instance in the demonstration of the difference between the blood of human beings and of anthropoids.

TABLE IX.

0.2 cc. of various dilutions of normal sera was added to 0.05 cc. of precipitin for chicken serum. Readings were made after 1 hour at room temperature.

Chicken serum diluted.						Guinea hen serum diluted.					
1:10	1:50	1:150	1:500	1:1,500	1:5,000	1:10	1:50	1:150	1:500	1:1,500	1:5,000
+±	++	+++	+±	+	±	+±	++	++	+	±	Tr.
Goose serum diluted.						Pigeon serum diluted.					
1:10	1:50	1:150	1:500	1:1,500	1:5,000	1:10	1:50	1:150	1:500	1:1,500	1:5,000
+±	+±	++	+±	±	Tr.	+	++	+±	+	±	0

The corpuscles of varieties or races within a given species showed much less difference, if any, as compared with those manifested by corpuscles of closely related species. Experiments under way would seem to indicate that the method may be usefully employed for estimating the degree of relationship of similar animals.

A source of error which has not been taken into account by some previous workers is to be found in the existence of differences in the blood corpuscles of individuals of the same species. A high serum titer for the blood of one individual may persist after absorption with the cells of another individual.[19] Errors due to such cause can only be excluded by the examination of a number of different specimens.

[19] Landsteiner, K., and van der Scheer, J., *J. Immunol.*, 1924, ix, 213, 221.

DISCUSSION.

The conception, based on the findings of Ehrlich and Morgenroth with agglutinins, that precipitins contain in general a multiplicity of antibodies, each acting on a single chemical group of the antigenic proteins, is not conclusively proven. Some authors[3,20] have even assumed that the antigenic proteins also have a complex composition, as that, for instance, the serum globulin of one animal will contain fractions specific for that species and certain others present in different species as well. This view as a general principle is contradicted by the known facts. Were it true, a hemoglobin of one source should crystallize in many different forms since hemoglobin behaves serologically like other proteins.[21,17] But observation shows that this is not the case. On the other hand, the conception of a multiplicity of antibodies in the sense indicated above also meets with some difficulties, as reflection will show. One has to account not only for the differing behavior of a given immune serum against two antigens, but for the fact that precipitins in general show a maximum activity with the homologous protein, the reaction decreasing gradually in strength with the distance in the zoological scale[22] when other antigens are tested.

The results of precipitin reactions with antigen containing binding groups of known chemical constitution leave no doubt as to the fact that a single precipitin will regularly react with other substances if their chemical structure is sufficiently near to that of the homologous antigen.[14] The alternative idea, so widely accepted,—that a given immune serum which reacts on the homologous antigen A and also on antigen B derived from another species consists of antibodies specific for a common group of A and B and contains other antibodies specific for a group peculiar to A,— is not tenable in this case. It is reasonable, therefore, to assume that the affinity of antibodies for natural antigens is not absolutely restricted to a single substance, but can extend to other similar ones.

[20] Arrhenius, S., Immunochemie, Leipsic, 1907.

[21] See Landsteiner, K., *Kon. Akad. Wetensch. Amsterdam*, 1921, xxix, 1029; Heidelberger, M., and Landsteiner, K., *J. Exp. Med.*, 1923, xxxviii, 561.

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

Since the antibodies which develop in various animals after injection of antigens, or in one individual at different stages of immunization, vary with regard to affinity and specificity, it is probable that in a single immune serum, as well, several such fractions are present and may possibly be separated. But one may suppose that each of them has a definite range of affinity for diverse antigens and that the phenomena observed in partial saturation do not fundamentally depend on a multiplicity of antibodies.

In the hemagglutinin experiments, a considerable part of the antibodies combined only with the homologous antigen, even when two closely related species were examined. The question arises whether these antigens are simple species-specific proteins or whether other factors are concerned in their specificity. They may possibly consist of such proteins combined with other substances or groups whose structure, unlike that of the proteins themselves, is not in close correspondence with the zoological system.[23] Instances which support this view are to be found in Forssman's heterogenetic antigens and in the isoagglutinin groups of human beings and animals. The fact that hemagglutinins of normal sera react frequently upon the corpuscles of related species as strongly as upon those of distant ones points in the same direction. Furthermore, the existence of similar groups in the blood corpuscles of distant species has been demonstrated in a general way by the method of splitting the combination of agglutinin and blood.[24] When, for instance, rabbit blood is agglutinated by beef serum, the combination can be dissociated by heating to 40–50°C., and the resulting liquid agglutinates not only rabbit corpuscles but also goose and frog blood markedly.

If, on the other hand, the hemagglutinogens should prove to consist only of proteins, they may have structural peculiarities, since proteins generally show a continuous variation by slow degrees

[23] Mention should be made of the possibly analogous relations described by Avery and Heidelberger in their immunological studies of the cell constituents of pneumococci.

[24] Landsteiner, K., *Münch. med. Woch.*, 1902, xlix, 1905. *Cf.* Landsteiner, K., in Oppenheimer, C., Handbuch der Biochemie, Jena, 1910, ii, pt. 1, 410. Landsteiner, K., and Reich, M., *Z. Hyg. u. Infectionskrankh.*, 1907,–08, lviii, 213. *Cf.* Bailey, C.E., *Am. J. Hyg.*, 1923, iii, 370.

from one species to the related ones. This is exemplified by observations on crystalline hemoglobins.[25]

CONCLUSIONS.

The results of the partial saturation of precipitins with antigens related in derivation to the homologous one give no conclusive evidence of the regular existence in a single immune serum of multiple antibodies which act specifically on various chemical groups of the antigenic proteins. It seems possible to explain at least a part of the facts by the assumption that a single antibody will react to different degrees with several similar substances.

By the partial absorption of hemagglutinins with heterologous blood, specific fractions were obtained. By such means one may readily differentiate the blood of related species, even when precipitins show but little difference.

The peculiarities in specificity manifested by precipitinogens and agglutinogens suggest an essential difference in the chemical structures which determine the specificity of the two kinds of antigens.

[25]Reichert, E. T., and Brown, A. P., Carnegie Institution of Washington, Publication No. 116, 1909. Landsteiner, K., and Heidelberger, M., *J. Gen. Physiol.*, 1923-24, vi, 131.