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Paul Vohs Jr.
Iowa State University

Elizabeth A. Cerny
Coe College

A. O. Haugen
Iowa State University

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Naturally Occurring Agglutinins for Pheasant Red Blood Cells[1]

PAUL VOHS, JR.,[2] ELIZABETH A. CERNY[3] AND A. O. HAUGEN[4]

Abstract. Sera from 24 vertebrate species and 3 antisera specific for human type A, B and Rh-D cells, respectively, were tested for their ability to agglutinate red blood cells of ring-necked pheasants (*Phasianus* sp.). Normal sera were grouped on the basis of agglutination titer, and the majority were included in the moderate and strong groups. Differential agglutination was limited, however. Substantial variation in ability to agglutinate pheasant cells occurred among sera of individuals of the same species. The usefulness of natural sera tested appears limited in the investigation of blood group factors of ring-necked pheasants.

Agglutinins in normal sera of vertebrate animals were discovered in 1875 by Landois (Landsteiner, 1946). Landsteiner called these agglutinins natural antibodies and stated that they may often be used to demonstrate differences between closely related species and individuals of the same species. The possibility of obtaining specific agglutinating sera in quantity for use in immunogenetic studies of the ring-necked pheasant (*Phasianus* sp.) seemed just cause for initiating a survey for natural antibodies in sera of vertebrate animals.

METHODS

Blood was obtained from the various animals and allowed to clot. Serum extruded from the clot was centrifuged and stored at –20°F until needed.

Twelve pheasants were selected at random from a group of birds with heterogeneous backgrounds. Red blood cells were obtained for testing by puncturing either left or right brachial vein or right jugular vein and drawing the blood into a 10 cc syringe or vacutainer tube containing 1-2 cc Alsever's solution. Alsever's solution was used to prevent clotting, and plasma was removed following centrifugation. Cells were washed twice, and suspensions of red blood cells (2%) were prepared in Alsever's solution. Cell suspensions were refrigerated until used for tests or discarded after 4 days.

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[2] National Institutes of Health Predoctoral Fellow, Iowa State University, Ames.
[3] National Science Foundation Undergraduate Science Education Participant, Coe College, Cedar Rapids, Iowa.
[4] Professor, Leader, Iowa Cooperative Wildlife Research Unit, Iowa State University, Ames.

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Agglutination tests were conducted in plastic depression trays with 12 rows of 8 cups in each. Serum to be titered was serially diluted with saline (0.85%) by doubling dilutions from 1:2 to 1:128 (human antiserum was titered to end point). Cells from the same 12 birds were tested at all dilutions with each serum. Trays were agitated 1-2 minutes following addition of cells and allowed to stand 30 minutes at room temperature. Saline controls were used throughout the survey.

Agglutination reactions were determined at the end of 30 minutes and again at 4 hours. If hemolysis occurred in the initial test, the serum was heated 30 minutes at 56°C to inactivate complement.

Table 1. Sera of vertebrate species tested for natural agglutinins with affinity for red blood cells of 12 pheasants.

Source of serum		Agglutination end-point titer	
Common name	Scientific name	low (range)	high
Flathead catfish	*Pylodictis olivaris**	1:2	1:8
American eel	*Anguilla rostrata*	0	1:64
American eel***		1:2	1:16
American toad	*Bufo americanus*	0	0
Blanding's turtle	*Emys blandingi*	1:32	1:128**
Blanding's turtle***		1:8	1:16
Pond slider	*Pseudemys scripta*	1:64	1:128**
Pond slider***		1:16	1:32
Softshell turtle	*Trionyx muticus*	0	0
Rat snake***	*Elaphe obsoleta*	0	1:8
Pied-billed grebe	*Podilymbus podiceps*	0	0
Red-tailed hawk	*Buteo jamaicensis*	1:64	1:128
Domestic turkey	*Meleagris sp.*	0	0
Great horned owl	*Bubo virginianus*	1:8	1:128
Great horned owl***		1:2	1:8
Common crow	*Corvus brachyrhynchos*	1:2	1:16
Brown thrasher	*Toxostoma rufum*	0	0
Opossum	*Didelphis marsupalis*	0	1:4
White-tailed jackrabbit	*Lepus townsendi*	1:8	1:16
Cottontail 1	*Sylvilagus floridanus*	1:16	1:128
Cottontail 21		1:4	1:32
Cottontail 49		1:16	1:64
Cottontail 55		1:4	1:64
Cottontail 70		1:128**	1:128**
Cottontail 76		1:16	1:32
Eastern fox squirrel	*Sciurus niger*	0	0
Eastern chipmunk	*Tamias striatus*	0	1:8
Raccoon***	*Procyon lotor*	1:128**	1:128**
Cat A	*Felis domestica*	1:32	1:128**
Cat B		1:32	1:64
Horse	*Equus caballus*	1:64	1:128
Swine	*Sus scrofa*	1:128**	1:128**
Beef	*Bos taurus*	1:128	1:128**
Goat (domestic)	*Capra sp.*	1:128	1:128**
Human anti-A	*Homo sapiens*	1:64	1:128
Human anti-B		1:128	1:256
Human anti-D		1:128	1:256

* Scientific nomenclature after Blair, Blair, Brodkorb, Cagle and Moore (1957).
** Indicates highest dilution tested but not end-point titer.
*** Serum heated to 56°C for 30 minutes.

Absorptions were accomplished by placing serum and packed cells together in a test tube, shaking for 1 minute, and allowing the mixture to stand 30 minutes. The supernate was then tested for reactivity with homologous cells. Additional absorptions were conducted until all reactivity was removed for homologous cells. Absorbed serum was then tested against red blood cell suspensions from each of the 12 birds.

Results

Sera from 24 vertebrate species and 3 antisera specific for human type A, B and Rh-D cells, respectively, were tested for their ability to agglutinate red blood cells of pheasants (Table 1). Normal sera were obtained from individuals of the following groups: fish (3), amphibians (1), reptiles (4), birds (6) and mammals (12). Sera of 6 individual cottontail rabbits and 2 house cats were also included.

Normal sera of the species tested were grouped on the basis of agglutination titer into negative, slight, moderate and strong (Table 2). Most sera were included in the moderate and strong classes. Only six sera failed to agglutinate pheasant red blood cells. Five sera listed among the strong reactors agglutinated all cells tested at 1:128, and no end-point titer was reached.

Table 2. Normal vertebrate sera grouped according to highest end-point titer of agglutination occurring among red blood cells of 12 ring-necked pheasants.

Grouping	No reaction	Slight	Moderate	Strong
Range of titer for group	0	1:2-1.8	1:16-1:32	1:64-1:128+
	American toad	gray rat snake*	flathead catfish	American eel
	soft shelled turtle	great horned owl*	American eel*	Blanding's turtle
	pied-billed grebe	opossum	Blanding's turtle*	pond slider
	turkey (domestic)	eastern chipmunk	pond slider*	red-tailed hawk
	brown thrasher		crow	great horned owl
	eastern fox squirrel		white-tailed jackrabbit	cottontail 49
			cottontail 21	cottontail 55
			cottontail 76	cottontail 70
				cottontail 1
				raccoon
				cat A
				cat B
				horse
				swine
				beef
				goat
				human anti-A
				human anti-B
				human anti-Rh D

* Heated to 56°C for 30 minutes to destroy complement.

The degree of differential agglutination among the 12 pheasants tested was limited (Table 3). Only four sera (group II) differentially agglutinated cells of individual pheasants to the extent that three doubling dilutions separated the end-point titers of the strongest and weakest reacting birds. Separations of two doubling dilutions occurred with five sera (group I). The majority of the sera showed little difference in end-point titers.

Table 3. Normal vertebrate sera grouped according to amount of differential agglutination occurring among red blood cells of 12 pheasants.

Group	I	II
Number of doubling dilutions between highest and lowest end-point titer	2	3 or more
	American eel* gray rat snake crow cottontail 21 eastern chipmunk	American eel great horned owl cottontail 55 cottontail 1

* heated to 56°C for 30 minutes.

Two (American eel and great horned owl) of the three sera which showed greatest differential agglutination caused hemolysis of pheasant cells at low titers and were heated to inactivate complement. The heating reduced differential agglutination of great horned owl serum to only one doubling dilution and American eel serum to two dilutions.

Substantial variation in ability to agglutinate pheasant cells occurred among sera of individual cottontails. The end-point titers for cells with least reactivity varied from 1:4 (21 and 55) to greater than 1:128 (Table 1). The highest end-point titer for 21 and 76 was 1:32 while serum from 70 exceeded 1:128. When the sera were grouped according to the amount of differential agglutination occurring among the cells of the 12 birds, cottontail sera were variable (Table 3).

Heating of the natural sera to inactivate complement reduced the end-point titers of most of the sera (Table 1). In some cases heating also reduced the ability of the serum to differentially agglutinate red blood cells of individual pheasants (Table 3).

A limited number of trial absorptions were made with some of the sera. The results were inconclusive, but removal of all reactivity resulted in most cases.

Discussion

Generalizations were limited by the survey techniques used, but some trends were evident. No phylogenetic relationships could be seen among individuals of the species whose serum did or did not agglutinate pheasant cells. Members of most of the phyla tested were found among each group.

The majority of normal sera tested was not suitable for use as reagents for differentiating between individual pheasants. Excluding those for which end-point titers were not reached, only the four antisera placed in group II showed promise. Heating of two of these sera to inactivate complement reduced their ability to differentiate to a level below group II. American eel serum showed most promise for further study, however. It was the only serum which failed to agglutinate all of the pheasant

cells at low titers while agglutinating cells of some of the pheasants. The results of agglutination tests with this serum were repeatable.

Cottontail rabbit serum showed wide variations in ability of normal serum of any one species to react with pheasant red blood cells. The results were in close agreement with Landstiener's (1946) conclusions that the level of natural antibodies varies greatly between individuals of the same species.

The usefulness of natural sera tested in the investigation of blood group factors of ring-necked pheasants appeared limited. Agglutinin titers of normal sera were very weak when compared with isoimmune and heteroimmune agglutinins produced in response to injections of pheasant red blood cells. Specificity of agglutination reactions was much reduced using normal sera, and the agglutinins lacked thermostability at temperatures necessary to inactivate complement.

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Food Habits of the Yellow Bass, *Roccus mississippiensis*, Clear Lake, Iowa, Summer 1962[1]

RUDY KRAUS[2]

Abstract. Return of Clear Lake water levels to normal was accompanied by some increase in growth of yellow bass in 1962. Entomostracans, chironomids and *Hyalella* sp. were the principal foods of young yellow bass. Immature insects, principally Diptera, were the predominant foods of older bass with cladocera, *Hyalella* sp. and copepods next in importance. There was no increase in utilization of small fish as food. Yellow bass were more active and fed more at night than in daytime.

INTRODUCTION

The yellow bass, *Roccus mississippiensis* (Jordan and Eigenmann), probably introduced into Clear Lake in the late twenties (Bailey and Harrison, 1945), has prospered so well that it is now

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[2] Loras College, Dubuque, Iowa.