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Species Specific Blood Groups in *Cynopithecus niger* (Celebes Ape).* (30012)

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The creation of primate research centers throughout the country has led to an increased interest in blood groups of primates, particularly as they relate to man. Aside from study of human blood groups, much of the early work was concerned with study of the antigenic structure of erythrocytes of Old World monkeys, particularly the species *Macaca mulatta* (rhesus monkey). This work has been reviewed recently by Wiener(1). Investigations were directed toward revealing factors related to human blood group factors, and it was through these investigations that the Rh_0 (D) factor of human red cells was discovered. More recently, investigations have centered on the extent of human blood group factors in anthropoid apes. Relatively few studies have been concerned with intra-species differences among lower primates with the exception of *M. mulatta*(2,3). This report will present data revealing species-specific blood factors present on red cells of the adult Celebes ape *(Cynopithecus niger)*.

A systematic survey of blood for species-specific blood groups in the lower primates using rabbit immune sera, after appropriate absorptions, was undertaken at the Oregon Regional Primate Research Center in 1962 (4). Attention was focused on the reactions of anti-rhesus erythrocyte serums absorbed with blood cells of the homologous species or of the Celebes ape. Suggestive intra-group differences among rhesus monkeys and among Celebes apes were noted, particularly in the latter, but these differences were not marked and the results were not easily reproducible. After a period of inactivity in this area of investigation, we undertook to reevaluate and expand the work previously done in this laboratory.

Materials and methods. Antisera were prepared by injecting rabbits with red cells from 5 selected apes; each rabbit was injected with cells from a single donor. Two series of injections were given. The first series consisted of 2 injections of 0.5 ml of a 50% suspension of cells given 5 days apart. The second series was given 5 weeks later and consisted of an intraperitoneal injection of 0.5 ml followed by an intravenous injection 2 days later. Four weeks later the animals were given a booster dose by intraperitoneal injection and were bled out 5 days later. Serums were separated from clotted cells, inactivated for 30 minutes at 56°C and stored in the freezer after addition of sodium azide to a final concentration of 0.1%.

Titrations of all serums were performed using human red cells of various ABO types, rhesus red cells and Celebes ape red cells. In addition, 2 serums were titrated using erythrocytes from 2 baboons (Papio species), 1 pig-tailed macaque *(Macaca nemestrina)*, 1 Sykes monkey *(Cercopithecus mitis)*, 3 tree shrews *(Tupaia glis)*, and 6 animals from 2 species of lemurs (*Lemur catta* and *Lemur fulvus*). Titrations were performed making 2-fold dilutions in saline in a volume of 0.05 ml and adding 1 drop of 2% suspension of test cells. Saline agglutinations were read after incubating for 15 minutes at room temperature followed by centrifugation for 30 seconds at 3500 rpm. One drop of bromelin

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was added to some serum-cell mixtures, incubated for 10 minutes and read after centrifugation for 30 seconds at 3500 rpm.

For preparation of specific antiserums for detection of isoantigens, serums were absorbed first with human cells, then with rhesus cells. Equal volumes of undiluted serum and washed packed red cells were allowed to incubate for one hour, centrifuged, and the supernatant fluid removed and stored. Generally it required 2 absorptions to remove all or nearly all agglutinins for the heterologous rhesus cells. For final absorption with Celebes ape cells, sera previously absorbed with human and rhesus red cells were diluted 1:5 and a portion of each antiserum was absorbed with cells from one or another of the 5 apes used for donor cells for immunization. Equal volumes of diluted antiserum and washed packed red cells were incubated for 1 hour at room temperature, centrifuged, and the supernatant fluid removed and stored. All absorbed serums found specific for one factor were pooled and used for testing cells of the 32 Celebes apes in the colony, 15 rhesus monkeys, and 9 samples of human red cells. Each pool of antiserum was used at 2 dilutions, 1:10 and 1:40. Bromelin was added to serum-cell mixtures; agglutination rarely occurred in saline. If agglutination failed to occur at either dilution with bromelin, the cells were retested by the antiglobulin technique using antiserum to whole rabbit serum prepared in goats. The antiglobulin serum was first absorbed with ape red cells in the cold. In addition human cells prepared by treatment with activated papain by the method of Stern *et al*(5) were used, and other tests were performed with cells suspended in 15% albumin.

Results. Titers against human cells were low, 10-50 units per 0.05 ml, whereas titers against rhesus cells overlapped in some cases those obtained with Celebes ape cells. These titers ranged from 160 to 2,560 units per 0.05 ml for the Celebes ape and 160-650 units for rhesus cells. Generally, however, titration of any one serum resulted in titers at least one doubling dilution higher for Celebes ape cells than for rhesus cells. Titers of 2 antisera tested with pig-tailed macaque cells were comparable to those obtained with the rhesus macaque, that is, 320 to 640 units per 0.05 ml. Absorption with baboon cells removed antibody to about the same extent for both species. Absorption of anti-ape serum with rhesus cells removed virtually all the antibody reacting with baboon cells. Titers against the prosimian species varied with the species. For example, the average titer against tree shrew cells was 40 units per 0.05 ml, that against *Lemur fulvus* was greater than 100, and that against *Lemur catta* was 50 units per 0.05 ml. Absorption twice with baboon cells reduced the titers, but absorption appeared to be less effective than with the more closely related species.

We felt that strong cross-reacting antibody might complicate the use of the partially absorbed serums for determining different isoantigens on the cells of the Celebes ape, therefore, we subjected the antiserums to systematic absorptions to remove all non-species specific antibody, first with human cells, then with rhesus cells. Absorption with human red cells of any ABO group, either Rh positive or Rh negative, removed antibody reacting against all human red cells. This absorption reduced the titer only slightly for rhesus and Celebes ape cells. A second absorption with rhesus red cells removed most or all of the antibody for rhesus cells, but not for Celebes ape cells. The final titers against rhesus cells ranged from negative to 5 or 10 units while that against Celebes ape cells remained at 80 units or greater. Antibody cross-reacting with rhesus cells agglutinated cells strongly in saline; antibody specific for Celebes ape cells reacted only weakly in saline, but induced strong and unequivocal agglutination when bromelin was added to the serum-cell mixture. The antigenic factor shared by Celebes apes 531 and 557 was designated as Cn_1 (*Cynopithecus niger* 1); the antigenic factor shared by apes 530, 540 and 557 was designated as Cn_2. Table I shows the results of these absorptions and the factors revealed by them. From these results we postulate the existence of 2 factors which, found singly or in combination, form 3 types. Type *Cn1* has factor Cn_1 only, *Cn2* has factor Cn_2 only, *Cn1,2* has both factors Cn_1 and Cn_2. None of the animals tested was found to lack both factors. No rhesus

TABLE I. Blood Factors Revealed by Absorptions of Anti-Ape Serums with Ape Cells.

Anti-serum	Test cells and factors present: 530 Cn_1	531 Cn_2	540 Cn_1	550 Cn_1	557 $Cn_{1,2}$
531/530*	—	+++	—	—	+++
531/550	—	+++	—	—	+++
531/557	—	—	—	—	—
531/540	—	+++	—	—	+++
540/530	—	—	—	—	—
540/531	+++	—	+++	+++	+++
540/550	—	—	—	—	—
540/557	—	—	—	—	—
557/530	—	+++	—	—	+++
557/531	+++	—	+++	+++	+++
557/540	—	+++	—	—	+++
557/550	—	+++	—	—	+++
530/531	+++	—	+++	+++	+++
530/540	—	—	—	—	—
530/557	—	—	—	—	—

* Antiserum and cells used for absorption are indicated by: No. of the antiserum/No. of animal donating cells for absorption.

or human cells were agglutinated by the 1:10 dilution of antiserum either in saline alone or with bromelin added. All ape cells were agglutinated at the 1:10 dilution of one or the other serum with bromelin added; those that failed to agglutinate at a 1:40 dilution with bromelin were retested by the antiglobulin method. All Celebes ape cells showing agglutination at 1:10 were agglutinated at a 1:40 dilution of serum by this method. Distribution of types is shown in Table II. Additional methods were used for testing the human cells with specific anti-ape antiserums. No reactions occurred at any dilution with tests performed in saline at 4°C, 22°C or 37°C, with activated papain at 37°C, or with albumin or bromelin at 37°C.

Discussion. Simpson(6) classifies the genera *Macaca, Cynopithecus, Cercocebus, Papio, Comopithecus, Mandrillus, Theropithecus, Cercopithecus, Allenopithecus* and *Erythrocebus* under one subfamily, *Cercopithecinae,* indicating their close relationship. Whether or not each of these should be considered a separate genus is in dispute. The existence of an antigen common to the Celebes ape, rhesus monkey, pig-tailed monkey and baboon is apparent although probably differing in specific antigenic groupings from species to species. It is possible that more than one antigen is responsible for the cross reactions obtained between anti-Celebes ape serum and the prosimian and human red cells.

TABLE II. Distribution of Blood Types in Celebes Apes.

Species	No. tested	Blood types: Cn_1	Cn_2	$Cn_{1,2}$
Celebes ape	32	12	1	19
Rhesus	15	0	0	0
Human*	9	0	0	0

* All human cells tested were of type O with all of the Rh, MNSsP, Kell, Duffy, Kidd, Lutheran and Lewis factors represented among the 9 test cells.

Although cross reaction occurred with human cells of all ABO types and cells of other closely related species when tested with unabsorbed serum, absorbed serums reacting with some but not all individuals of the species *Cynopithecus niger* failed to react with any human or rhesus cells tested. It appears then that antigenic blood factors are present on the red cells of the Celebes ape that are not shared by at least one closely related species, *M. mulatta.* The small size of the colony and incomplete knowledge of the troop or area from which the individuals came precluded study of population genetics or mode of inheritance. However, studies are under way to determine the antigenicity of the Celebes ape cells in the heterologous species, *M. mulatta.* Should cross-immunization prove feasible, breeding experiments will be attempted with the hope of obtaining an experimental model for erythroblastosis fetalis studies.

The methods developed for detection of the isoantigens of the Celebes ape erythrocytes have been found useful in similar studies in rhesus monkeys. Incomplete investigations reveal that isoantigens are present on cells of the rhesus monkey.

Summary. Specific isoantigens, designated Cn_1 and Cn_2, have been detected on erythrocytes of the Celebes ape, thus defining 3 types of individuals, Cn1, Cn2, and Cn1,2. Methods have been developed which may prove useful in detection of isoantigens among other primate species.

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Experimental Enteric Infection of Chickens with an Avian Adenovirus (Strain 93).* (30013).

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Man is now known to be host to enteric infections with a multiplicity of viral agents. The pathogenesis of such infections, particularly at the tissue level, and the roles of humoral and cell-associated factors in the subsequent immune response are not fully understood. Although "orphan" viruses have been isolated from the gastrointestinal tract of many animal species(1), considerations of animal size and cost plus the difficulties of maintaining stock free of interfering wild viruses have rendered these systems unsuitable as experimental models for many investigators.

An earlier paper(2) described some properties of an avian adenovirus, strain 93, isolated in chick kidney tissue culture from the cloacal contents of a market chicken. The present paper reports experiments undertaken to study the behavior of strain 93 in its natural host and in particular to determine whether it might serve as a useful and convenient model for enteric viral infection with characteristics analogous to those observed in the infection of man with the human enteric viruses.

Materials and methods. Experimental animals. White Leghorn eggs were obtained from a local poultry farm and hatched in the laboratory. The chicks were fed a commercial growing mash.

Virus. Strain 93 was propagated in chick kidney tissue culture. Birds were inoculated in various experiments with 0.1 ml of undiluted tissue culture fluid containing virus at the third through eleventh passage levels. The material was introduced into the esophagus by means of a gelatin capsule.

Specimens. Throat cultures were obtained by rubbing a moistened swab over the walls of the posterior pharynx and cloacal samples by inserting a swab into the organ. Ten per cent suspensions of tissue in Hanks' basic salt solution were homogenized with a Virtis micro-assembly unit and the tissue extracts collected after low speed centrifugation. One hundredth ml-0.08 ml of bile was diluted in 3 ml of Hanks' solution. Serum for viral isolation or antibody titration was obtained from blood drawn by cardiac puncture. The processing of specimens, passage in chick kidney tissue culture for viral isolation and titration of serum antibody have been described(2).

Identification of virus isolates. All viral isolates from tissue and a sampling of those recovered from throat and cloacal cultures were typed in a standard neutralization test against 20 antibody units of anti 93 rabbit serum. Additional throat and cloacal isolates were typed by hemagglutination-inhibition (HI) against 4 units of immune chicken serum.

Results. Excretion and pathogenesis following primary oral challenge. The susceptibility of chicks to infection by the oral route was studied in 6 one-day-old chicks fed $10^{6.33}$ $TCID_{50}$ of 93 virus. Cloacal and throat swabs were taken on days 1-7, 10, 12, 14 and 17 post-inoculation. Blood for serum anti-

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