known to occur in congestive heart failure, may contribute to the reduction (*5*). This view is supported by the finding of a reduction of renal norepinephrine concentration in those animals in which the left ventricular concentration was most strikingly reduced. In addition, it must be considered that interference with the binding or synthesis of norepinephrine, or both, may be involved. It is clear that the operative procedure itself does not mechanically interfere with innervation of the heart; no significant change in the cardiac norepinephrine stores occurred in the sham-operated animals.

In 8 of the 13 animals with congestive heart failure due to a 2-mm aortic constriction, the concentrations of norepinephrine in the left ventricle were reduced to between 5 percent and 19 percent of the normal mean value. These changes are comparable with those achieved with certain pharmacologic agents whose anti-adrenergic action is mediated through their ability to deplete neurotransmitter stores (*6*). Therefore, the possibility must be considered that the profound decrease in cardiac norepinephrine which occurs in congestive heart failure may be associated with an abnormality in adrenergic function. The very important role of the cardiac sympathetic nerves in increasing myocardial function is well documented (*2*). In the heart in which myocardial performance is already impaired, interference with such an important compensatory mechanism could contribute to further deterioration of cardiac function. Thus, it is possible that these studies provide an approach to the understanding of an important aspect of the congestive heart failure state.

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Blood Groups of Chimpanzees: Demonstrated with Isoimmune Serums

Abstract. *Of five chimpanzees injected with blood of other chimpanzees in Freund's adjuvant, three produced antiserums for simian blood factors designated* $\mathbf{A}^c$, $\mathbf{B}^c$, *and* $\mathbf{C}^c$, *respectively. Factors* $\mathbf{A}^c$ *and* $\mathbf{B}^c$ *determine four blood groups which were distributed among 60 chimpanzees in conformity with the triple-allele hypothesis. Factor* $\mathbf{C}^c$ *appears to belong to an independent blood group system. All three simian blood factors are distributed independently of sex. An antithetical relation exists between the simian blood factor* $\mathbf{A}^c$ *and blood factor* $\mathbf{N}^v$, *the latter being a blood factor shared by all human N cells. Since the simian blood factors* $\mathbf{A}^c$ *and* $\mathbf{B}^c$ *are also related, the three blood factors* $\mathbf{A}^c$, $\mathbf{B}^c$, *and* $\mathbf{N}^v$ *must belong to one and the same blood group system, which is named the V-A-B blood group system of chimpanzees. The V-A-B blood group system of chimpanzees appears to be the counterpart of the M-N-S blood group system of man.*

Studies on blood groups of apes and monkeys have so far been carried out almost exclusively with reagents originally prepared for typing human blood (*1*). With such specific antiserums, we have investigated numerous species of nonhuman primates during the past two years (*2*). Another method of study is by immunizing laboratory animals with simian red cells (*3*), a technique successful for demonstrating blood groups in rhesus monkeys (*4*). It has been postulated (*3*) that by immunization with red cells of closely related species, as well as by isoimmunization, antibodies may be produced which demonstrate individual differences within the species. In fact, individual differences in chimpanzee red cells have been demonstrated with chimpanzee antiserums to human red cells (*5*).

In the present investigation, five chimpanzees were each injected intramuscularly in multiple sites with whole chimpanzee blood mixed with an equal amount of complete Freund's adjuvant (*6*). Each chimpanzee received the blood of a single donor selected at random. The injected animals all developed large abscesses which persisted for 6 months or longer. For convenience the animals were bled after 6 or 7 months, at which time three of them had isoantibodies in their serums. All three antiserums gave different reactions and were therefore arbitrarily designated as anti-$\mathbf{A}^c$, anti-$\mathbf{B}^c$ and anti-$\mathbf{C}^c$, respectively (*7*). The superscript "c" for chimpanzee has been added to the symbols, in order to distinguish these antiserums from blood-grouping reagents currently being used for other species. The three antiserums detect three corresponding blood factors, $\mathbf{A}^c$, $\mathbf{B}^c$ and $\mathbf{C}^c$, which we designate as "simian" blood factors, because in contrast to the human-like blood factors described in our previous papers, these blood factors were initially detected in apes with isoimmune serums.

The antiserums to factors $\mathbf{A}^c$ and $\mathbf{B}^c$ reacted clearly only by the antiglobulin technique at 37°C. For these tests, rabbit antiserum to human blood serum, prepared for human blood-grouping tests, was used, after further absorption with chimpanzee red cells. The chimpanzee antiserums to $\mathbf{A}^c$ and $\mathbf{B}^c$ factors also reacted by the saline agglutination method, but those reactions were much weaker and were poorly reproducible. The anti-$\mathbf{C}^c$ reagent reacted by the antiglobulin technique, but the titer was higher by the ficinated cell technique, which proved not to be suitable for the anti-$\mathbf{A}^c$ and anti-$\mathbf{B}^c$ reagents. None of the serums gave clear reactions by the acacia or albumin techniques or in tests at refrigerator temperature, nor were they isohemolytic.

A series of 60 chimpanzees from the large primate colony maintained at the Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia, has been included in the present investigation. All have been tested not only for the simian blood factors $\mathbf{A}^c$, $\mathbf{B}^c$, and $\mathbf{C}^c$, but also with anti-$\mathbf{N}^v$ lectin (*Vicia graminea*), as well as with the usual A-B-O, M-N, and Rh-Hr human typing reagents. The pertinent findings are summarized in Table 1.

Theoretically, the four factors, $\mathbf{N}^v$,

Table 1. Distribution of the blood factor $\mathbf{N}^{v}$ and the simian blood factors $\mathbf{A}^{e}$, $\mathbf{B}^{e}$, and $\mathbf{C}^{e}$ among 60 chimpanzees.

Serial number	Blood factors				No. of chimpanzees		Binary number*
	$\mathbf{N}^{v}$	$\mathbf{A}^{e}$	$\mathbf{B}^{e}$	$\mathbf{C}^{e}$	Group A	Group O	
1	−	−	−	−	0	0	0000
2	−	−	−	+	1	0	0001
3	−	−	+	−	1	0	0010
4	−	−	+	+	5	1	0011
5	−	+	−	−	8	0	0100
6	−	+	−	+	4	0	0101
7	−	+	+	−	6	0	0110
8	−	+	+	+	3	0	0111
9	+	−	−	−	2	0	1000
10	+	−	−	+	1	1	1001
11	+	−	+	−	12	0	1010
12	+	−	+	+	7	0	1011
13	+	+	−	−	5	1	1100
14	+	+	−	+	0	0	1101
15	+	+	+	−	1	0	1110
16	+	+	+	+	1	0	1111

* This arrangement according to the binary system may prove useful also for purposes of coding.

$\mathbf{A}^{e}$, $\mathbf{B}^{e}$, and $\mathbf{C}^{e}$, could determine 16 blood types, as shown in Table 1. The blood types have been arranged in the table in what seemed to be the most logical order, according to the reactions of the red cells. As can be seen, with this arrangement the reactions given by the red cells correspond to the number, expressed in the binary system, equal to one less than its serial number in the table. Of the 16 possible combinations or blood types all but two have been encountered among the 60 chimpanzees tested. The blood types found are not evenly distributed among the 60 chimpanzees, since some types are common while others appear to be quite rare. In fact, as many as half of the combinations, namely serial numbers 1, 2, 3, 8, 9, 14, 15, and 16, include only 9 of the 60 chimpanzees. The striking symmetrical distribution of the types is not merely accidental, but is due to certain relationships among the four blood factors, as was shown by statistical analysis.

Two by two contingency tables show, first, that the simian blood factors $\mathbf{A}^{e}$ and $\mathbf{B}^{e}$ are not independent. (For $\mathbf{A}^{e}$ and $\mathbf{B}^{e}$, $\chi^2 = 13.4$, $n = 1$, $P < .0005$. With the Yates correction, χ^2 becomes 11.5, and $.0005 < P < .001$.) Factors $\mathbf{A}^{e}$ and $\mathbf{B}^{e}$ determine four blood groups distributed as follows: Group O^{e} (with red cells lacking $\mathbf{A}^{e}$ and $\mathbf{B}^{e}$ blood factors) is equal to 8.3 percent; group A^{e} is equal to 30.0 percent; group B^{e} is 43.3 percent; and group $\mathrm{A}^{e}\mathrm{B}^{e}$ is 18.3 percent. If these four groups are inherited by triple allelic genes, like the four A-B-O groups, the same formulas can be used for calculating the gene frequencies (*8*). These prove to be $O^{e} = 28.9$ percent; $A^{e} = 33.0$ percent; and $B^{e} = 43.0$ percent; so that the sum of the calculated gene frequencies is approximately 105 percent. The deviation of this sum from 100 percent is about equal to its standard error, so that these results support the hypothesis of triple allelic genes. Thus, the simian blood factors $\mathbf{A}^{e}$ and $\mathbf{B}^{e}$ appear to be part of the same blood group system. On the other hand, 2×2 contingency tables indicate that $\mathbf{C}^{e}$ is independent of both $\mathbf{A}^{e}$ and $\mathbf{B}^{e}$, so that the simian blood factor $\mathbf{C}^{e}$ probably belongs to a different blood group system of chimpanzees.

A 2×2 contingency table for the blood factors $\mathbf{N}^{v}$ and $\mathbf{A}^{e}$ gave the most striking results, namely that when either factor is present the other tends to be absent (see Table 1). In this case, $\chi^2 = 15.3$, for 1 degree of freedom, so that $P < .0001$. This establishes a relationship between the simian blood factor $\mathbf{A}^{e}$ and the blood factor $\mathbf{N}^{v}$ shared by all human N cells. Surprisingly, however, the contingency table for blood factors $\mathbf{N}^{v}$ and $\mathbf{B}^{e}$ gives $\chi^2 = 0.68$, and $P = 0.40$, approximately. This suggest that $\mathbf{N}^{v}$ is independent of $\mathbf{B}^{e}$ though antithetical to $\mathbf{A}^{e}$, even though, as has been shown, $\mathbf{A}^{e}$ and $\mathbf{B}^{e}$ belong to the same blood group system.

The paradox has been resolved as follows. Instead of three allelic genes, O^{e}, A^{e}, and B^{e}, one postulates (*10*) six allelic genes, O^{v}, O^{v} A^{v}, A^{v}, B^{v}, and B^{v}, where O^{v} and O^{v} are subdivisions of gene O^{e} (comparable to the subdivision of the human gene A into A^{1} and A^{2}), and, similarly, genes A^{v} and A^{v} are subdivisions of $\mathbf{A}^{e}$, while B^{v} and B^{v} and subdivisions of $\mathbf{B}^{e}$. The presence of a large V superscript in the gene symbol signifies that the gene gives rise to an agglutinogen with the blood factor $\mathbf{N}^{v}$; the presence of a small v superscript indicates the absence of blood factor $\mathbf{N}^{v}$ in the corresponding agglutinogen. Calculation (10) of the six gene frequencies from the distribution of the types among the 60 chimpanzees yields the following: $O^{v} = 0.155$; $O^{v} = 0.120$; $A^{v} = 0$; $A^{v} = 0.315$; $B^{v} = 0.182$; and $B^{v} = 0.228$. Thus, every or almost every A^{e} gene determines an agglutinogen $\mathbf{A}^{e}$ lacking blood factor $\mathbf{N}^{v}$; in contrast about half the O^{e} and half the B^{e} genes determine agglutinogens with factor $\mathbf{N}^{v}$, and half without. This accounts for the seemingly paradoxical results described above. Thus, blood factors $\mathbf{A}^{e}$, $\mathbf{B}^{e}$, and $\mathbf{N}^{v}$ belong to one and the same blood group system, which is designated the V-A-B blood group system.

The 2×2 contingency test indicates that $\mathbf{N}^{v}$ and $\mathbf{C}^{e}$ are probably independent. Furthermore, blood factors $\mathbf{N}^{v}$, $\mathbf{A}^{e}$, $\mathbf{B}^{e}$, and $\mathbf{C}^{e}$ are distributed independently of sex. There are too few chimpanzees of group O (only three) in the present series to permit any conclusions regarding any possible relationship between these blood factors and the A-B-O blood groups.

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6. Complete Freund's adjuvant, Difco Laboratories.
7. To avoid ambiguity, symbols for blood factors are printed in boldface type, symbols

for genes are printed in italics, and symbols for agglutinogens, phenotypes, and blood group systems are printed in regular type.

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Rubella Virus: Inhibition in vitro by Amantadine Hydrochloride

Abstract. *Amantadine (or 1-adamantanamine) hydrochloride, a compound reported to be active against influenza viruses and Sendai virus, inhibited the growth of rubella virus in tissue culture. The antiviral activity appears at an early phase of the infection and is not due to direct inactivation of the virus.*

Amantadine hydrochloride (1-adamantanamine hydrochloride, EXP 105-1) inhibits influenza (A, A_2, and C) and Sendai viruses in vitro and in vivo (*1*). Although only a limited number of viruses from the rather extensive group examined were susceptible, nevertheless we tested the activity of amantadine hydrochloride against certain other viruses and observed that rubella virus was inhibited in vitro.

Continuous cultures of monkey kidney cells of the LLC MK_2 line (*2*) were grown and maintained with Medium 199 (*3*) containing 1 percent horse serum. Cell cultures, untreated or treated with amantadine hydrochloride (*4*), were infected with the WM strain of rubella virus (*5*), and at appropriate times samples were withdrawn and titrated. Rubella virus was assayed by its capacity to interfere with the development of the more easily recognizable, typical cytopathology seen after superinfection with 100 tissue-culture 50-percent infective doses ($TCID_{50}$) of ECHO-11 virus (*6*) applied as previously described (*5*).

Judged by visible microscopic morphology, LLC MK_2 cells could tolerate amantadine hydrochloride at a concentration of 31.2 μg/ml but not 62.5 μg/ml. In view of the complex, indirect method of assaying rubella virus by its capacity to interfere with ECHO-11 cytopathology it was also necessary to establish that ECHO-11 virus was not inhibited by amantadine hydrochloride. Typically, in 4 days an inoculum of approximately 100 $TCID_{50}$ of ECHO-11 virus grew equally in the presence or absence of amantadine hydrochloride. The final $TCID_{50}$ was $10^{-7.5}$ in the presence of 31.2 μg of amantadine hydrochloride per milliliter compared with $10^{-7.3}$ in control cultures.

Data for three separate experiments showing the inhibitory effect of amantadine hydrochloride on the growth of rubella virus in vitro are presented in Table 1. Drug was added just before inoculation of the cell cultures with rubella virus. A period of 5 hours was allowed for virus adsorption before the cells were washed and the medium was replaced to maintain appropriate concentrations of inhibitor. Samples for titration were taken at 0, 3, and 6 days, these times being based on the growth characteristics of rubella virus described elsewhere (*7*). Concentrations of amantadine hydrochloride inhibiting virus growth did not have detectable direct action against free rubella virus when tested after a contact period of 12 hours. Since the time these data were presented (*8*) the finding has been confirmed elsewhere (*9*). It should also be mentioned that, although the results in Table 1 are presented as interference with the cytopathogenic effect of ECHO-11 virus, inhibition of the less obvious cytopathology directly caused by rubella virus, which has also been observed (*10*), parallels the interference findings. Thus the inhibition of rubella virus demonstrated in Table 1 appears not to be an artifact of the indirect assay system.

The influence of time of treatment on the antiviral effectiveness of amantadine hydrochloride was also studied (Table 2). Results are given for two separate experiments showing that when added during the first 3 hours amantadine hydrochloride clearly inhibits replication of rubella virus. When treatment was initiated 5 or more hours after virus inoculation, no protection was observed; this suggests that, as is the case with influenza virus (*1*), amantadine hydrochloride may be inhibiting rubella virus in vitro at an early stage in the infectious process. On the other hand the compound failed to inhibit Chang's strain of lipovirus or the Edmonston strain of measles virus (*11*).

Amantadine hydrochloride appears to have a fairly circumscribed range of antiviral activity embracing several types of influenza virus (*1*), Sendai virus (*1*), and rubella virus. This evidence, as well as its size (*12*) and nucleic acid species (*7*), suggests that rubella virus is related to the myxoviruses. Also amantadine hydrochloride may possibly prove effective in the chemotherapy of rubella as a disease.

Table 1. Amantadine hydrochloride (EXP 105-1) inhibition of rubella virus in tissue culture. Virus yields from three experiments are expressed as negative $\log_{10}$ of the 50 percent interfering dose (Int. D_{50}) (*5*, *6*). Designated concentrations of drug were added before rubella virus and maintained thereafter. After a virus adsorption period of 5 hours cultures were washed, and the medium including drug was replaced.

Day	Rubella virus yield (neg. $\log_{10}$ Int. D_{50})		
	EXP 105-1		Untreated
	31.2 μg/ml	15.6 μg/ml	
0	<1, 1, 2*	1, <1, 2*	<1, 1, 1.8*
3	<1, 1, 1*	1.8, 1, 2.3*	2.3, 2, 2.5*
6	1, 1.3, <1*	3, 1.8, 2.7*	3.3, 3.5, 3.5*

* Virus inoculum was not removed.

Table 2. Effect of delayed addition of amantadine hydrochloride (31.2 μg/ml) at various times after rubella virus infection. First samples were removed at the indicated time of drug addition. Values are given for two experiments, a and b.

Time of addition (hr)	Rubella virus yield (neg. $\log_{10}$ Int. D_{50})			
	First sampling		96 hr	
	a	b	a	b
Control (5 hr)	1.5,	1.0	3.3,	4.0
0	1.3,	1.7	1.5,	1.5
1	1.5		2.0	
3	1.3		1.5	
5	1		3.3,	3.0
10, 12	1.3,	1.5	4.0,	3.3
24		1.7		3.3
36		2.0		3.5
48		2.5		3.3

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