Agglutination of African Primate and Rodent Erythrocytes by Adenoviruses, Reoviruses, and Enteroviruses

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African nonhuman primate and rodent erythrocytes were tested for agglutination by adenoviruses, reoviruses, and enteroviruses. Squirrel erythrocytes were agglutinated by reovirus serotypes and adenovirus types 3, 11, 16, and 21. Adenoviruses also agglutinated brazza monkey erythrocytes to the same titers as those obtained with either rhesus or grey monkey cells. Prototype reovirus types 1 and 2 agglutinated grey monkey erythrocytes to much lower titers than either squirrel or human group O red cells. Among the enteroviruses tested, only echovirus types 7 and 12 agglutinated grey, red-tail, brazza, and rhesus monkey erythrocytes. The specificity of agglutination of squirrel, grey, and brazza monkey erythrocytes by reoviruses, echoviruses, and adenoviruses, respectively, was confirmed by hemagglutination-inhibition tests. The titers obtained were similar to those observed with erythrocytes usually used in these tests. Erythrocytes of bush babies, potto unstriped grass mice, swamp rat, rusty-nosed rat, bush rat, harsh-furred mice, soft-furred rat, and giant rat were not agglutinated by adenoviruses, reoviruses, or enteroviruses.

Rosen (7) first demonstrated agglutination of mammalian erythrocytes by adenoviruses. Subsequent studies showed that adenoviruses could be divided into four groups on the basis of agglutination of monkey and rat erythrocytes. Nowadays, hemagglutination-inhibition (HI) tests are routinely used to type adenoviruses. Dardoni and Zaffiro, cited by Papadimitriou (6), showed that the three types of reoviruses agglutinated human group O erythrocytes. Later, Gomas and Tamh (3) reported that only reovirus type 3 agglutinated bovine red blood cells (RBCs). More recently, Munube (5) demonstrated that reoviruses are capable of agglutinating grey monkey (Cercopithecus aethiops) erythrocytes, although to lower titers than human group O RBCs. This has been confirmed by testing RBCs from 20 individual grey monkeys. Hemagglutination of human group O erythrocytes by some types of echoviruses was demonstrated by Goldfield (2). In 1962, Bussell, Karzon, and Hall (1) in their extensive study of echoviruses found that echovirus type 7 and one strain of echovirus type 12 could agglutinate rhesus monkey (Macaca mulatta) red cells.

From the available literature, it appears that little or no study has been done on the agglutination of (i) African rodent erythrocytes by adenoviruses and reoviruses and (ii) African monkey erythrocytes by enteroviruses. The findings reported in this paper are part of a continuing study on the possible use of African primate and rodent erythrocytes in further characterization of certain adenoviruses, reoviruses, and enteroviruses.

MATERIALS AND METHODS

Animal species. The animal species whose erythrocytes were tested included primates: grey monkey (Cercopithecus aethiops), rhesus monkey (Macaca mulatta), red-tail monkey (Cercopithecus ascanius), brazza monkey (Cercopithecus neglectus), bush babies (Galago senegalensis and G. demidovii), and potto (Perodicticus potto); rodents: striped ground squirrel (Xerus erythropus), unstriped grass mice (Arvicanthis abyssinicus and Lemniscomys striatus), swamp rat (Otomys irroratus), rusty-nosed rat (Oenomys hypoxanthis), bush rat (Athomys rusei), harsh-furred mice (Lophronomys flavopunctatus), soft-furred rat (Pranomyx jacksoni), giant rat (Cricetomys gambiae), and albino rat (Rattus norvegicus). Each

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virus was tested against erythrocytes obtained from at least five members of the above animal species, except for the brazza monkey and the potto, where only one individual was available.

**Erythrocytes.** Blood was collected in three volumes of Alsever solution and washed three times in physiological saline (0.85%). The washed erythrocytes were stored as a 10% suspension in Alsever solution at 4°C until used, usually within 7 days.

**Viruses.** Adenoviruses, echovirus types 3, 4, 6, and 12, and reovirus 1 (Lang), 2 (Jones), and 3 (Dearing) were obtained from the East African Virus Research Institute stock of viruses. Local reoviruses and echoviruses used were isolated from children before a mass polio virus vaccination campaign was commenced. Other viruses used were kindly supplied by I. Domok, Head, World Health Organization (WHO) Virus Study Team stationed at the East African Virus Research Institute, Entebbe, Uganda. Adenoviruses were grown in HeLa cells, and other viruses in primary grey monkey kidney cell monolayers.

**Sera.** Prototype antisera used for HI were obtained from the National Center for Disease Control, Atlanta, Ga., U.S.A. Rabbit, monkey, and human sera used in reovirus HI test were obtained from the Virus Research Institute stock of sera.

**Hemagglutination tests.** The tests were done in standard (WHO) perspex plates. Two-tenths-milliliter twofold dilutions of the virus suspensions in 0.85% saline were made in a series of wells. To each well was added 0.2 ml of 1% erythrocyte suspension in normal saline. A cell control consisting of diluent and erythrocyte suspension was included. Parallel tests were investigated after incubation at 4, 22, and 37°C. The tests were read when cell controls settled down into fine “buttons,” usually after 1 to 1.5 hr.

**HI tests.** The HI tests were carried out by the technique of McCrae and Barton (4). Five-tenths milliliter of a 1:5 dilution of serum in 0.85% saline was mixed with an equal volume of 25% acid-washed kaolin and allowed to stand at room temperature for 30 min. The mixture was then centrifuged. The supernatant fluid was removed and heat-inactivated at 56°C for 30 min. To 1 ml of supernatant fluid, 0.1 ml of 50% RBC suspension was added, incubated at 4°C for 1 hr, and then centrifuged. The supernatant fluid contained serum at 1:10 dilution. Two-tenths-milliliter serial twofold dilutions of the supernatant fluid were made in the wells of a perspex plate. An equal volume of 4 hemagglutinating units of virus was added to each well. After shaking, the plates were incubated for 1 hr at 37°C, and then 0.2 ml of 1% erythrocyte suspension was added. The tests were incubated at 4°C until cell controls settled. Both virus and serum controls were included. The last well to show complete inhibition was recorded as the end point.

**RESULTS**

**Agglutinination.** Squirrel erythrocytes were agglutinated by reoviruses, but not by enteroviruses (Table 1). Reovirus type 3 agglutinated squirrel erythrocytes to titers two to three times greater than human group O erythrocytes; RBCs from other animal species tested failed to be agglutinated by this virus. The high sensitivity of squirrel RBCs for reovirus type 3 was further demonstrated by agglutination of one local virus strain; this strain also did not agglutinate either human group O or bovine erythrocytes. Erythrocytes from the grey monkey were agglutinated by only prototype reovirus types 1 and 2 which had been multiply passaged in tissue culture cells, and to lower levels than squirrel or human O erythrocytes.

Among the enterovirus types tested, only echovirus types 7 and 12 agglutinated grey and red-tail monkey erythrocytes to almost the same titers as human group O RBCs (Table 1). Local strains of ECHO 7 and 12 also agglutinated grey and red-tail monkey cells. On the other hand titers obtained with rhesus and brazza monkey erythrocytes were somewhat lower. Rhesus monkey RBCs were agglutinated by the prototype strain of ECHO 7, but not by other enteroviruses.

Adenovirus types 3, 11, 16, and 21 agglutinated brazza monkey RBCs. Hemagglutination titers with these erythrocytes were comparable to titers obtained with rhesus and grey monkey RBCs. Although squirrel erythrocytes were also agglutinated by these adenovirus types, the titers were too low for practical usage.

In addition, erythrocytes from a number of animal species tested failed to be agglutinated by adenoviruses, enteroviruses, and reoviruses. These included potto and bush babies among primates and unstriped grass mice, swamp rat, rusty-nosed rat, bush rat, harsh-furred mice, soft-furred rat, and giant rat among rodents.

**Hemagglutination-inhibition.** HI titers obtained when hyperimmune sera were tested with representative echoviruses and adenoviruses by using human group O, grey, or brazza monkey erythrocytes were similar. Reovirus types 1 and 2 HI antibody titers with hyperimmune chicken and randomly selected animal or human sera were no different from those found with human group O and squirrel cells.

**DISCUSSION**

The agglutination of squirrel erythrocytes by reoviruses is of particular interest. Generally, reoviruses agglutinated squirrel erythrocytes to higher titers than human group O cells, and the erythrocytes were readily agglutinated by both prototype and local strains of reovirus type 3, some of which failed to agglutinate human
### Table 1. Hemagglutination by adenoviruses, enteroviruses, and reoviruses

| Virus          | Hemagglutination titers with the following erythrocytes: |
|---------------|----------------------------------------------------------|
|               | Human group O | Grey monkey | Red-tail monkey | Rhesus monkey | Brazza monkey | Ground squirrel | Albino rat |
| Adeno 3       | ND            | 256<sup>a</sup> | -<sup>b</sup>    | 256           | 512           | 4                | -          |
| Adeno 11      | ND            | 128          | -                | 64            | 64            | 4                | -          |
| Adeno 16      | ND            | 256          | -                | 256           | 256           | 8                | -          |
| Adeno 21      | ND            | 128          | -                | 64            | 128           | 16               | -          |
| Adeno 10      | ND            | -            | -                | -             | -             | -                | 256        |
| Adeno 24      | ND            | -            | -                | -             | -             | -                | 256        |
| Adeno 26      | ND            | -            | -                | -             | -             | -                | 16         |
| Adeno 27      | ND            | -            | -                | -             | -             | -                | 256        |
| Adeno 1       | ND            | -            | -                | -             | -             | Partial          | -          |
| Adeno 2       | ND            | -            | -                | -             | -             | -                | Partial    |
| Adeno 12      | ND            | -            | -                | -             | -             | -                | -          |
| Coxsackie B-5 | 64            | -            | -                | -             | -             | -                | ND<sup>c</sup> |
| Echo 3        | 128           | -            | -                | -             | -             | -                | ND         |
| Echo 6        | 256           | -            | -                | -             | -             | -                | ND         |
| Echo 7        | 128           | 64           | 64               | 32            | 32            | -                | ND         |
| Echo 7 (SG238)<sup>d</sup> | 64       | 64           | 64               | -             | -             | -                | ND         |
| Echo 7 (SG1428) | 256      | 256          | 256              | -             | -             | -                | ND         |
| Echo 11       | 128           | -            | -                | -             | -             | -                | ND         |
| Echo 12       | 256           | 128          | 256              | 16            | -             | -                | ND         |
| Echo 12 (SG21627) | 32      | 32           | 32               | -             | -             | -                | ND         |
| Echo 13       | 64            | -            | -                | -             | -             | -                | ND         |
| Echo 19       | 256           | -            | -                | -             | -             | -                | ND         |
| Reo 1 (Lang)  | 128           | 32           | -                | -             | -             | -                | 256        |
| Reo 1 (Hung)<sup>e</sup> | 128     | 8            | -                | -             | -             | -                | 256        |
| Reo 1 (SG20654) | 32       | -            | -                | -             | -             | -                | 64         |
| Reo 2 (Jones) | 1,024         | 64           | -                | -             | 1,024         | -                | ND         |
| Reo 2 (Hung)  | 256           | 32           | -                | -             | 256           | -                | ND         |
| Reo 2 (SG19867) | 16      | -            | -                | -             | 16            | -                | ND         |
| Reo 2 (SG20133) | 128     | -            | -                | -             | 128           | -                | ND         |
| Reo 3 (Dearing) | 8         | -            | -                | -             | 32            | -                | ND         |
| Reo 3 (Hung)  | 8             | -            | -                | -             | 64            | -                | ND         |
| Reo 3 (SG19886) | -         | -            | -                | -             | 8             | -                | ND         |

<sup>a</sup>Titers are expressed as the reciprocal of the final dilution of virus suspensions giving complete hemagglutination.

<sup>b</sup>-: Negative (titers were less than 1 in 2).

<sup>c</sup>ND: Not done.

<sup>d</sup>SG: Uganda field strains.

<sup*e</sup>Hung, Hungary, prototype strains supplied by I. Domok.

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The finding that echovirus types 7 and 12 agglutinated grey, red-tail, and brazza monkey erythrocytes adds further to the spectrum of erythrocytes that react with these viruses. Busel et al. found that prototypes ECHO 7 and 12 and one local strain ECHO 7 could agglutinate rhesus monkey red cells, but to low titers; our results are in agreement. Rhesus monkey erythrocytes were poorly agglutinated by some ECHO 7 and 12. On the contrary, both prototype and local strains of these viruses agglutinated grey and red-tail monkey cells without fail. Further tests with additional strains are required.

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LITERATURE CITED

1. Bussell, R. H., D. T. Karzon, and F. T. Hall. 1962. Haemagglutination and haemagglutination-inhibition studies with echo viruses. J. Immunol. 88:39.
2. Goldfield, M., S. Srihonges, and P. J. Fox. 1957. Haemagglutinins associated with certain human enteric viruses. Proc. Soc. Exp. Biol. Med. 96:788.
3. Gomatos, P. J., and I. Tamm. 1962. Reactive sites of reovirus type 3 and their interaction with receptor substances. Virology 7:455.
4. McCrae, A. D., and M. B. Barton. 1965. Haemagglutination in the typing of enteroviruses. Bulletin, Ministry of Health and Public Health Laboratory Service (Britain), 24:357.
5. Munube, G. M. R. 1967. Agglutination of grey monkey (Cercopithecus aethiops) erythrocytes by reoviruses. East Afr. Virus Res. Inst. Ann. Rep. no. 17.
6. Papadimitriou, J. M. 1966. An electron microscopic study of reovirus haemagglutination. Aust. J. Exp. Biol. Med. Sci. 44:701-704.
7. Rosen, L. 1958. Haemagglutination by adenoviruses. Virology 5:574-575.