Reports and Letters

Blood Volume of the Rhesus Monkey

In the course of an investigation of the hemopoietic response of the rhesus monkey to total-body x-irradiation, it became necessary to express data in terms of the average blood volume of these animals. A review of the literature failed to reveal this information and the study reported here was undertaken to establish this value.

Twenty prepuberty rhesus monkeys, 10 males and 10 females, were used in the study. For a period of 6 wk prior to the determinations, the animals were maintained on a standard laboratory diet. During this period the animals did not exhibit any sign of the diseases common to the species.

The blood-volume determinations were made according to the method of Aust *et al.* (*1*), using radioactive iodinated human serum albumin (IHSA) as the tracer agent. In brief, 2.0 μc of IHSA in a volume of 0.5 ml was injected into the right femoral vein of each animal. Ten minutes later, a sample of blood was withdrawn from the left femoral vein into a heparinized syringe. A 1.0-ml aliquot of this sample was then assayed in a well-type scintillation counter. Previously, 0.5 ml of the IHSA had been diluted to a total volume of 1000 ml. A 1.0-ml aliquot of this known dilution was used as a standard and was assayed in the same manner. The blood volume was computed by the following formula:

$$\frac{\text{Net counts/min of standard} \times 10^3}{\text{Net counts/min of sample}} = \text{B.V.}$$

The 20 animals included in this study weighed from 2.2 kg to 5.3 kg. The blood volumes ranged from 49 ml/kg to 71 ml/kg. The average blood volume was found to be 60.9 ml/kg, and the probable error of this mean is 0.96 ml/kg. This distribution about the mean closely corresponds to values given by Freinkel *et al.* (*2*) and Storaasli *et al.* (*3*) in similar studies performed on human beings.

Although human serum albumin is a foreign protein to these animals, the half-time of the plasma clearance of the IHSA was found to be 22 hr, and this approximates values reported by Storaasli (*3*) in human beings. In addition, repeated injections of this material into individual monkeys have not induced demonstrable foreign protein reactions.

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Identification of Vaccine Strains of Newcastle Disease Virus

Before random purity and potency tests can be applied to living virus vaccines for Newcastle disease, a practical method is needed for typing and identifying the strain of virus in the vaccine (*1*). Such a method based on several years of study of strains of virus from the Newcastle disease repository at Wisconsin (*2*) has been tested successfully, using samples of vaccines submitted by the Biological Products Section of the U.S. Department of Agriculture.

The vaccine sample is reconstituted so that 100 chicken vaccine doses are contained in 1 ml, and five tests are made on selected dilutions of the sample: LD_{50} for 10-day chicken embryos; mean death time of the minimum lethal dose; intracerebral pathogenicity for day-old chicks; susceptibility to neutralization by Newcastle disease virus antiserum of known titer; and bacterial contamination (*3*). Allanto-amniotic fluids harvested from embryos inoculated for the LD_{50} test are examined for equine erythrocyte agglutinins and for resistance of the fowl erythrocyte agglutinins to inactivation at 56°C.

The strains are typed according to their growth rate (*4*), which is measured by the mean death time of the minimum lethal dose. The lentogenic, or slowly growing, strains, which take 90 to 150 hr to kill embryos, include only a few strains that are all of low pathogenicity for chickens. Two strains are used in intranasal or aerosol vaccines. The mesogenic, or intermediate, strains, which take 60 to 90 hr to kill, include a larger group of strains possessing low to high pathogenicity for chickens. Two strains are used in wing-web vaccines. The velogenic, or rapidly growing, strains, which kill in 40 to 60 hr, include a large group of strains of moderate to high pathogenicity for chickens. All are unsatisfactory as vaccines.

The strain is defined by the use of additional test characteristics. A comparison (Table 1) of the four widely used American living virus vaccine strains—Bl, LaSota, Roakin, and MK107 (L), illustrates the use of definitive tests (tests 6 and 7) in conjunction with typing tests (tests 2 and 3).

The LD_{50} in test 1 is dependent on vaccine production procedure, such as the quantity of diluent and buffers used in packaging, and is not of diagnostic importance. The intracerebral pathogenicity index of lentogenic strains should be less than 0.25, so the index (test 3) helps to confirm the test for the mean death time (test 2). The capacity to agglutinate equine erythrocytes (test 6) differentiates the two strains in each type. Stability of the fowl erythrocyte agglutinins at 56°C (test 7) serves as an additional check on the strain definition, since all four strains should be inactivated in 15 min.

Some of the afore-described tests have been made on all of the 80 strains of Newcastle disease virus available in the repository, and other tests have been made on fewer of these strains. Twelve additional characteristics are being con-

Table 1. Characteristics of four vaccine strains

Sample	Test 1, LD_{50} (log)	Test 2, mean death time (hr)	Test 3, intracerebral pathogenicity (index)	Test 6, equine hemagglutinin (index)	Test 7, resistance 56°C (min)	Diagnosis	
						Type	Strain
A	7	120	0.1	0	> 15	Lentogenic	Bl
B	7	100	0.1	0.4	> 15	Lentogenic	LaSota
C	5	70	1.1	0	> 15	Mesogenic	Roakin
D	5	60	1.1	0.5	> 15	Mesogenic	MK107 (L)

sidered and are particularly useful in differentiating velogenic strains (*5*). Studies on the genetic stability of certain test characters (*6*) indicate that, with proper precautions, the method suggested here for strain typing and differentiation can be successfully employed.

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References and Notes

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Conditioned Aversion to Saccharin Resulting from Exposure to Gamma Radiation

It was previously observed (*1*) that the water and food consumption of rats was depressed during exposure to a relatively low dose of low-intensity gamma radiation. The severity of this depression increased with successive exposures to radiation, although consumption between exposures was similar to or exceeded that of nonirradiated controls. It was suggested that the progressive change in consummatory behavior during repetitive exposure may be, in part, a conditioned response in which the avoidance of water and food is strengthened by learning through repeated coupling with the radiation situation.

A clearer demonstration of the learning phenomenon would be provided if the conditioned avoidance of water or food could be elicited in the absence of radiation exposure. This report (*2*) describes the results of an experiment designed to provide such a test. The water available during irradiation was made discriminative to the animal by the addition of saccharin. Subsequently, the saccharin flavor was employed as a taste stimulus in a postirradiation test for a conditioned aversion to the discriminative fluid.

The animals were Sprague-Dawley stock bred at this laboratory. Each animal was maintained, in an individual cage. Two plastic 100-ml drinking bottles with glass nipples were attached to each cage except during specified periods. The difference in weight of each drinking bottle and of its contents before and after a given test period was used as the estimate of fluid consumption.

Eighty male rats from 16 litters were subjected to a preirradiation series of tests to determine their degree of preference of a 0.1-percent saccharin solution in relation to tap water. Both fluids were presented concurrently. Tests were also made to evaluate a possible position preference in drinking in the two-bottle situation. Very few animals exhibited a drinking-bottle position preference strong enough to overcome their preference for saccharin. As a result of these tests, 20 animals with the lowest preference for the saccharin solution and/or the highest position preference were eliminated. The remaining animals were randomly assigned to six groups, with the restriction that each group contain at least one member, and not more than two members, from each litter. The experimental treatment for each group is shown in Table 1.

For radiation exposure, all animals were confined in Lucite boxes and exposed in the gamma field of a 7-c cobalt-60 radiation source. Attached to each box was a single plastic drinking bottle that contained either tap water or saccharin solution, as specified in the experimental design. The 30-r and 57-r exposures were made at radiation intensities of 5.0 r/hr and 9.5 r/hr, respectively. The sham-irradiated groups were placed in the radiation field behind a lead shield. The total exposure period was 6 hr for all groups.

Measurements of fluid consumption

Table 1. Experimental treatment for each group.

Group	No. of animals	Radiation exposure dose (r)	Fluid present during exposure
I	10	0	Tap water
II	10	0	Saccharin solution
III	10	30	Tap water
IV	10	30	Saccharin solution
V	10	57	Tap water
VI	10	57	Saccharin solution

Table 2. The median saccharin solution preference score for each group during the initial 48-hr postirradiation test period

Radiation dose (r)	Groups II, IV, VI (saccharin present during irradiation)		Groups I, III, V (water present during irradiation)	
0	88.0	(11.0)*	93.2	(2.3)
30	33.4	(9.8)	94.8	(8.2)
57	3.1	(3.7)	96.8	(1.8)

* Standard error of the median.

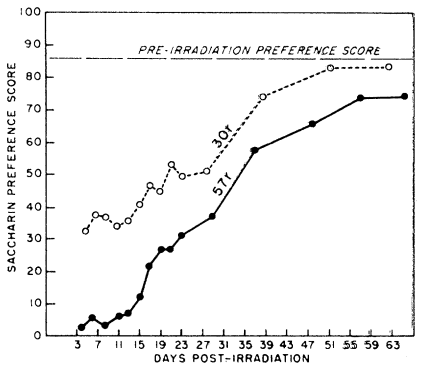

Fig. 1. Median saccharin preference scores for animal groups exposed concurrently to gamma radiation and saccharin-flavored drinking water.

indicated that all animals had tasted the fluid presented to them during radiation exposure. On day 1 postirradiation, the groups that had received water during exposure were presented with the saccharin solution only for 6 hr, and the other groups received water only in order to equalize the experience with saccharin. On day 2 postirradiation, saccharin solution and water were simultaneously available to each animal for a 6-hr period. Beginning with the day 3 postirradiation, both saccharin solution and water were available to each animal on a continuous basis. Measurements of consumption were made at 24-hr intervals.

During the postirradiation preference testing period, the drinking bottles were reversed daily to avoid a stereotyped position response. An effect of position was not detectable when the saccharin preference was distinctively high or low. However, when the scores were in the neutral range of preference, a position effect could be detected. In order to minimize the effect of position on the scores, each consecutive left and right measurement pair are combined for each animal, and 48-hr values are reported.

The saccharin preference score utilized is the quotient for the ratio

$$\frac{\text{Saccharin solution intake (g)}}{\text{Total fluid intake (g)}} \times 100$$

The median preirradiation saccharin score for all animals was observed to be 86.1 (S.E. = 1.0) for two 6-hr test periods; that is, the saccharin solution constituted 86.1 percent of the total fluid consumption. The results of the initial postirradiation preference test period for each group are summarized in Table 2. Animals presented with saccharin during the sham-irradiation (0 r) maintained their preference during the postirradiation test period. However, animals that were exposed to 30 r or 57 r with saccharin available during irradiation exhibited a marked decrease in their pref-