rubella virus. Untreated control cultures were included in the experiment.

It is apparent that FUDR did inhibit vaccinia virus as expected, and that thymidine did reverse the inhibitory effect of the drug since by the 6th day the infectious yield was comparable to the untreated control. The yield of rubella was unaltered.

Since completion of this work, Cusumano (9) independently found that 5-IUDR (Iododeoxyuridine) failed to inhibit the yield of rubella virus in tissue culture. The evidence reported that 5-FUDR does not inhibit the growth of rubella virus indicates that rubella virus is an RNA virus. Purification of rubella virus for direct chemical analysis will be the proof of the type of nucleic acid of the virus.

Summary. Rubella virus grows to a maximum titer of approximately $10^{-5.0}$ Int.$D_{.50}$ in LLC-MK_2 cell cultures, with 2:1 input in multiplicity. There is always cell-associated virus which accounts for a small portion of the infectious yield. 5-FUDR did not affect in any way the yield of rubella virus as compared to the untreated control. Rubella virus therefore appears to belong to the RNA viruses.

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Effect of an Alpha-2 Globulin Fraction on Antibody Formation in Rabbits and Mice.* (29596)

HANS L. SPIEGELBERG† AND WILLIAM O. WEIGLE‡

Scripps Clinic and Research Foundation, Division of Experimental Pathology, La Jolla, Calif.

It has recently been reported by Mowbray that an alpha-2 globulin fraction obtained by DEAE cellulose chromatography called "fraction C" has the ability to prolong homograft rejection in rats(1) and to prevent antibody formation in rabbits(2). Treatment with fraction C was effective when started either before or with, but not after, injection of antigen. It was therefore suggested that fraction C may interfere with the induction phase of antibody production(2).

Fraction C contains a number of alpha-2 globulins and possesses RNAse as well as phosphatase activity(2,3). It could be extracted from the thymus, but not from other organs tested(3). Preparations obtained from both homologous and heterologous serum have been reported to be equally effective.

The experiments reported here were undertaken to study further the effect of bovine fraction C on antibody formation in rabbits and mice.

Materials and methods. Animals. Male

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† Research Fellow in Division of Exp. Pathol., Scripps Clinic and Research Foundation, La Jolla, Calif.

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New Zealand rabbits, weighing 2 to 3.5 kg and male Swiss Webster mice, weighing 20 to 25 g were used in the present experiments.

Fraction C. Fraction C was prepared from bovine serum as described by Mowbray(2). Bovine blood was allowed to clot at room temperature for 2 to 4 hours. Centrifugation of the serum and all further procedures were performed at 4°C. The serum was diluted 1 to 2.5 with 0.03 M acetic acid. The resulting mixture had a pH of 5.0. The precipitated euglobulin was removed by centrifugation and the supernatant applied to a DEAE cellulose column (77 × 9.5 cm, holding 400 g DEAE cellulose "Selectacel standard," Brown Co., Berlin, N.H.) previously equilibrated with 0.03 M acetate buffer, pH 5.0. The column was successively eluted with 0.03, 0.1 and 0.2 M acetate buffer, pH 5.0 until the effluent showed an O.D. reading at 280 mμ of less than 0.02. Fraction C was then eluted with 0.5 M acetate buffer, pH 5.0 and concentrated by pressure dialysis. The first 4 preparations were dialyzed against buffered saline pH 7.2 and stored at —20°C. Three additional preparations were dialyzed against distilled water and lyophilized. Ninety to 120 mg of fraction C were obtained per liter of serum. Analysis by paper electrophoresis showed that all preparations contained a major alpha-2 component and a small amount of an alpha-1 component, representing 5-9% of the total protein.

One preparation of fraction C was obtained from an extract of 2.5 kg beef thymus according to Mowbray(3). The final preparation contained 270 mg protein.

RNAse determination. A semiquantitative method to determine RNAse activity was used(4). Equal volumes of 2% melted agar and 2% RNA (Nutritional Biochemicals Co.) in 0.1 M acetate buffer, pH 5.0, were mixed and 20 ml aliquots poured into petri dishes and allowed to solidify. Wells of 5 mm diameter were cut and filled with 0.1 ml of the test solution containing 10 mg protein per ml. The petri dishes were incubated at 37°C overnight for 18 hours. Ten ml of a 5% TCA solution was then layered over the agar and the clear zone around the well representing degraded RNA was measured.

Antigens. Human serum albumin (HSA), Squibb lot No. 1186 1RR, was trace labeled with I^{131} (I*) according to the method previously described(5). Rat erythrocytes were washed and labeled with Cr^{51} by incubating the erythrocytes at 37°C for 30 minutes with $NaCr^{51}O_4$ (Rachromate Abbott).

Administration of fraction C. Fraction C was injected intraperitoneally (i.p.) into mice and rabbits, except in one experiment, where it was given intravenously.

Immune elimination. Rabbits were injected intravenously (i.v.) with 10 mg of I*HSA and the rate of disappearance was measured by counting in a whole body scintillation counter. Mice were injected i.v. with 0.1 ml per 10 g bodyweight of a 25% suspension of Cr^{51} labeled rat erythrocytes. 0.05 ml blood was drawn daily with a calibrated pipette from the retroorbital venous plexus, the cells lysed in 1 ml H_2O and the radioactivity determined in a well type scintillation counter. All counts were corrected for background, decay and coincidence.

Antibody determination. Rabbits were bled 3 days after the immune elimination of the I*HSA was completed and the sera were stored at —20°C. Precipitating antibody to HSA was measured by a quantitative precipitin technique employing I*HSA(6).

Results. Rabbits. The effect of bovine fraction C on the immune elimination of HSA in rabbits is summarized in Table I. Twenty-eight rabbits in 3 different experiments were injected with 10 mg I*HSA. Eight of the rabbits were injected with 100 mg of fraction C on days 0 and 3 as recommended by Mowbray(2)(Exp. 1) and 8 were injected with 200 mg of fraction C on days 0, 3 and 6 (Exp. 2 and 3). All animals showed immune elimination of I*HSA. Precipitating antibodies to HSA could usually be detected in their sera. Two rabbits, one in the control group and one in a group which received fraction C, gave an immune elimination of I*HSA but precipitating anti-HSA could not be detected in their sera. There was no significant difference in the time of immune elimination and in the amount of

TABLE I. Effect of Fraction C on Antibody Production to HSA in Rabbits.

Treatment	Exp No.	Fraction showing immune elimination	Day of immune elimination	μg Antibody N anti-HSA/ml serum
.15 M NaCl	1	4/4	10.7	11.1
.15 M NaCl	2	4/4	9.8	13.2
.15 M NaCl	3	4/4	13.7	4.6
Total		12/12	11.7 ± 2.7†	9.7 ± 7.9†
Fraction C, 100 mg/rabbit on days 0* and 3 (i.p.)	1	8/8	9.3	10.0
Fraction C, 200 mg/rabbit on days 0,* 3 and 6 (i.p.)	2	4/4	11.5	13.2
Fraction C, 200 mg/rabbit on days 0,* 3 and 6 (i.v.)	3	4/4	14.2	5.0
Total		16/16	10.0 ± 3.0†	9.2 ± 5.9†

* Fraction C was given 30 min before injection of I* HSA on day 0. i.p., intraperitoneally; i.v., intravenously.
† Standard deviation.

anti-HSA between control and treated animals. No antibodies to fraction C were found in the sera from any of the rabbits by the agar double diffusion technique.

Mice. The elimination of rat erythrocytes from the circulation of untreated mice is shown in Fig. 1. During the first 2 days a slow non immune phase with a half survival time of approximately 3 days was always observed. At the third or fourth day a sudden change in the speed of elimination occurs and the rat erythrocytes are rapidly eliminated from the circulation. Immune elimination was completed in 69% of the control mice on day 3 and in the remaining mice on day 4. As can be seen in Table II, doses from 50 to 2000 mg per kg of fraction C prepared from bovine serum did not inhibit or delay the immune elimination of rat erythrocytes. Similarly, fraction C prepared from bovine thymus in doses of 250 and 500 mg per kg had no effect on the immune elimination of rat erythrocytes.

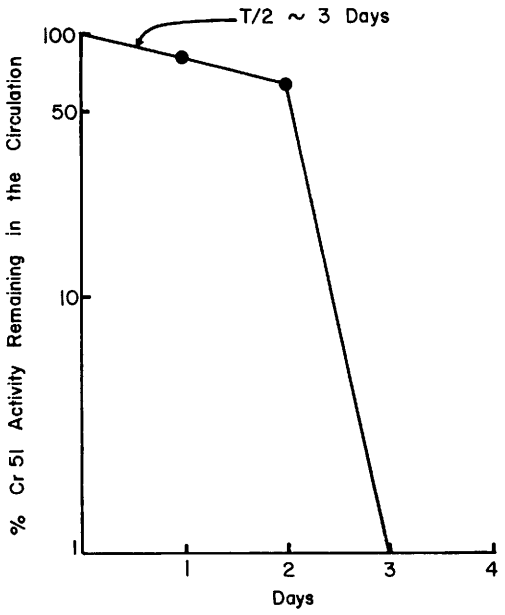

FIG. 1. Elimination of Cr^{51} labeled rat erythrocytes from circulation of mice.

RNAse activity of fraction C. In the semiquantitative test used for detection of RNAse activity all preparations of fraction C showed activity. As a control, 0.1 mg of commercial RNAse (Sigma Chemicals Co.) was included in each test, which gave a 10 mm zone of RNA degradation. One mg of different preparations of fraction C obtained from bovine serum gave a zone of approximately 3 mm. The fraction C prepared from the thymus extract showed a zone of 5 mm.

Discussion. The present studies have shown that the alpha-2 globulin fraction C isolated from bovine serum had no effect on antibody production either to human serum albumin in rabbits or to rat erythrocytes in mice. The results obtained with rabbits are in contrast to previous findings by Mowbray, who reported that fraction C prepared from bovine serum inhibited immune elimination of I*HSA in 6 out of 7 rabbits and sup-

TABLE II. Effect of Fraction C on Immune Elimination of Rat Erythrocytes in Mice.

Treatment	Time (hr) of inj of Fraction C*	Fraction showing immune elimination on Day 3	Day 4
NaCl	—	33/48 (68.7%)	48/48 (100%)
Fraction C prepared from bovine serum			
50 mg/kg i.p.	— 4, +24, +48	9/10	10/10
175 "	— 4, +24, +48	9/12	12/12
250 "	— 4, +24, +48	9/10	10/10
250 "	—24, — 6, — 2, +24, +48	3/3	3/3
500 "	— 4, +24, +48	1/2	2/2
500 "	—24, — 8, +24, +48	4/5	5/5
1000 "	— 4, +24, +48	4/10	10/10
2000 "	— 4, +24, +48	4/5	5/5
Total		43/57 (75.4%)	57/57 (100%)
Fraction C prepared from bovine thymus			
250 mg/kg	—4, +24, +48	4/5	5/5
500 "	—4, +24, +48	4/5	5/5
Total		8/10 (80%)	10/10 (100%)

* Rat erythrocytes were injected on day 0. Negative time units refer to injections before and positive time units after injection of rat erythrocytes.

pressed antibody formation to various serum protein antigens as well as to sheep erythrocytes in rabbits(2). The difference between his and the present results is difficult to explain. One possibility may be that the different findings are the result of a difference in the responsive state of the rabbits. It has frequently been observed in this laboratory, that rabbits obtained from various breeding sources showed marked differences in their response to protein antigens. Furthermore, our preparations of fraction C may have contained less of the active factor. Rabbits were therefore treated with 2 times and mice with 5 to 60 times the recommended dose of fraction C, however, these doses also, had no effect on antibody production. Other unknown factors may be responsible for the difference in the results reported by Mowbray and those obtained in the present study. In any event, the suppression of antibody formation by the alpha-2 globulin fraction C apparently is a phenomenon which cannot easily be duplicated.

Summary. The effect on antibody production of an alpha-2 globulin fraction which has been reported to inhibit antibody formation was studied in rabbits and mice. Rabbits were immunized with I^{131} labeled HSA and the immune elimination and antibody production to HSA determined. Mice were injected with Cr^{51} labeled rat erythrocytes and the immune elimination followed. No difference in the immune response was found in rabbits and mice between control animals and animals receiving the alpha-2 globulin fraction.

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