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Inhibitory Effect of Heparin on Rous Sarcoma Virus

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During the course of investigations on anti-Rous sarcoma virus (RSV) activity in human plasma, using the chick embryo cell (CEC) residual virus assay procedure of H. Rubin et al. (Proc. Natl. Acad. Sci. U.S. **47**:1058, 1961), we observed that many citrated plasma samples caused a twofold or greater inhibition of the Harris (HA) or Schmidt-Ruppin 2 (SR 2) strains of RSV, or both. Residual virus was not reduced, however, when the same samples were tested by the chorioallantoic membrane (CAM) assay method (V. Groupé et al., J. Bacteriol. **74**:409, 1957). A large number of heparinized chicken plasmas (41 of 51) behaved in this same manner when tested with HA-RSV. These discrepancies in plasma residual virus between the two assay systems suggested a nonspecific antiviral effect in the plasmas rather than the presence of RSV antibody. Further, since sera tested with the plasmas were not inhibitory to these two strains of RSV, it appeared that the antiviral activity might be related to the presence of the anticoagulants used in the study. Therefore, both anticoagulants were tested for anti-RSV activity by obtaining paired serum and heparinized or citrated plasma samples from chickens and testing the effect of each on RSV. Each component was incubated with virus for 40 min at 37 C, and residual virus was determined by CEC assay.

Heparinized plasma caused a marked inhibition of two of the four strains of RSV tested (Table 1). Schmidt-Ruppin RSV 2 titer was reduced 64 to 93% by all concentrations of heparin, and HA-RSV was similarly inhibited by a concentration of 80 units per ml or greater. Bryan standard (BS) and Prague (PR) strains of RSV were not affected by the heparinized plasma.

Harris and SR-RSV 2 titers were also compared by use of the CAM for residual virus assay. Little or no reduction was detected by this procedure; titers in plasma differed by less than 7% from the corresponding serum titers.

The effect of heparin on RSV in the absence of plasma was tested by incubating SR-RSV 2 in CEC growth medium containing 100 units per ml of heparin. The mixture was then diluted 1:10, and 0.1 ml was assayed on CEC. Virus titer was reduced 60% by the heparin. Cells treated with this same concentration of heparin at 18 hr before and 3 hr after infection with SR-RSV 2 were transformed to the same degree as control cells without heparin, suggesting that inhibition was the result of binding of heparin to the virus and not to an irreversible effect on the cells. The fact that the heparin effect was eliminated on the CAM and that not all strains were equally sensitive to heparin when assayed together on CEC from the same pool also suggests this mechanism. A reversible binding of virus to heparin has been reported with several strains of herpes simplex virus (A. J. Nahmias and S. Kibrick, J. Bacteriol. **87**:1060, 1964; Vaheri, Acta Pathol. Microbiol. Scand., Suppl. **171**:1, 1964). A concentration of heparin as low as 0.1 μg/ml of plating medium reduced the infectivity titer of the strain used by Vaheri; Nahmias and Kibrick found the minimal inhibitory concentration to be 10 to 20 μg/ml. In the present study, the lowest concentration of heparin employed was 20 units per ml of whole blood, equivalent after dilution to 0.05 units or 0.5 μg/ml of plating medium. This concentration was inhibitory to the SR 2 strain of RSV.

It is clear from these experiments that heparin inhibited some strains of RSV in the absence of antibody to these strains and at a concentration comparable to that used in obtaining plasma. According to the criteria for immune status proposed by H. Rubin et al. (Virology **17**:143, 1962), chickens in the present study would be classified antibody-positive from plasma analysis but negative by serum analysis. Therefore, the use of plasma for antibody determinations against some strains of RSV could lead to false-positive reactions, and should be avoided.

It appears also from this study that only RSV of subgroup B of the avian tumor virus classification of Vogt and Ishizaki (Virology **26**:664, 1965) and Vogt (*personal communication*) was inhibited. Subgroup A, represented by the BS-RSV, and a possible third subgroup, C, represented by the Prague strain, were not inhibited. Therefore, a "heparin marker" such as that suggested by Vaheri for herpes simplex and other viruses may be present in the Rous sarcoma viruses and perhaps in other avian tumor viruses.

TABLE 1. *Comparison of RSV titers in chicken serum and heparinized plasma*

Chicken no.[a]	Heparin concn[b]	RSV titers[c]			
		BS	SR	HA	PR
1	0	20.3	3.4	1.6	5.4
	20	24.0 (0.0)	0.9 (73.6)	1.1 (31.2)	6.6 (0.0)
2	0	24.0	3.4	1.7	6.3
	25	22.5 (6.0)	0.8 (76.5)	1.1 (35.2)	8.2 (0.0)
3	0	22.0	2.6	1.8	6.6
	40	21.4 (2.7)	0.4 (84.6)	1.0 (44.4)	6.3 (4.5)
4	0	22.1	2.8	1.8	6.3
	40	20.3 (8.0)	1.0 (64.3)	1.2 (33.3)	6.1 (3.0)
5	0	22.6	3.4	1.5	5.2
	80	20.9 (7.5)	0.5 (85.3)	0.4 (73.4)	4.8 (5.7)
6	0	20.2	4.2	1.8	6.1
	200	25.0 (0.0)	0.3 (92.9)	0.4 (77.8)	5.5 (10.0)
—[d]		22.2	4.8	1.5	7.6

[a] Each chicken was bled twice, first for serum and then for heparinized plasma. Chicken no. 1 was bled into a syringe containing a combination of sodium and potassium heparin, obtained from Scientific Products Co., Evanston, Ill. Sodium heparin from Organon, Inc., West Orange, N.J., was used for chickens 2 to 6.

[b] USP units per milliliter of whole blood.

[c] Titers in focus-forming units per milliliter, $\times 10^5$. Figures in parentheses indicate per cent reduction in virus titer in heparinized plasma compared with nonheparinized serum. The following abbreviations for Rous sarcoma virus strains were used: BS, Bryan Standard; SR, Schmidt-Ruppin 2; HA, Harris; PR, Prague.

[d] Antibody-negative serum controls.

The effect of citrate on RSV was not as marked. A slight reduction of 10 to 30% in titer was observed with SR-RSV 2 but not with HA-RSV at the citrate concentration tested (10^{-4} M in plating medium). Bryan standard and PR-RSV were not inhibited. At higher concentrations of citrate ($\geq 10^{-3}$ M), CEC growth was affected and all virus titers were lowered. The anti-RSV activity in the human plasmas may therefore be the result of a combination of citrate and heterogenous plasma effect on CEC. Additional studies are in progress to determine whether this is true.