son, Thayer and Kopp(1) using 440 μg of hydrocortisone found a liver glycogen deposition of 730 mg per 100 g of liver in the adrenalectomized rat. In the present study a total dose of 200 μg produced a glycogen deposition of 900 mg per hundred grams of liver. While the 2 experiments are not entirely comparable the results tend to indicate that the bird is no less responsive to glucocorticoids than the mammal.

The point that many investigators have failed to note is the fact that cortisone is virtually inactive as a glucocorticoid in the bird. Thus, the observation of Brown *et al.* must be restricted to cortisone. Certainly enormous doses of this hormone are required to elicit a physiological change in the bird. However, a failure to respond is not obtained for all glucocorticoids. Indeed, hydrocortisone and corticosterone are highly active in the bird. Therefore, it is important that studies involving the use of glucocorticoids in the bird employ corticoids which are physiologically active in the bird. This failure of the chick to respond in a manner comparable to that seen in the mammal has also been noted for the progestogens(13).

The depressant activity of testosterone upon blood glucose levels in the hen has previously been demonstrated(14). It appears that this effect is not a result of direct interference in carbohydrate metabolism of the bird but is, rather, an indirect effect resulting from an increase in hematocrit induced by treatment with testosterone.

Summary. The effects of a series of steroids on the carbohydrate metabolism of the bird were studied using blood glucose and liver glycogen as endpoints. The highly active compounds in the hyperglycemic reaction were hydrocortisone and corticosterone and the active compounds in the liver glycogen test were the above two plus 11-dehydrocorticosterone. Cortisone was inactive in both tests and 11-desoxycorticosterone acetate was tested only in the hyperglycemia reaction and was inactive. If hydrocortisone is assigned an activity of 100 in the glycogen test, then corticosterone shows an activity of 12.5 and 11-dehydrocorticosterone an activity of 1. In direct contrast to the mammal, cortisone is inactive in the bird. Testosterone appears to lower blood glucose but this apparently is a result of an increase in red blood cell number rather than an absolute drop in glucose present in the blood. Stilboestrol had no effect on blood glucose levels.

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Interaction of Myxoviruses with Human Blood Platelets *in vitro*. (26370)

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The interaction of myxoviruses with red blood cells has been the subject of extensive investigation. The kinetics of adsorption, its requirements of cations and the kinetics of elution are known(1). The distribution and chemical identity of the virus receptors

have also been established(2). The inability of the red blood cells to adsorb additional virus(3) is associated with a change in electrophoretic mobility of the red blood cell(4), and has been ascribed to loss of receptors due to the action of a viral enzyme(5). This enzyme is similar to the receptor destroying enzyme (RDE) produced in bacterial cultures(6). Myxoviruses have been shown to be adsorbed onto white blood cells, even in absence of cations(7). When leukocytes are treated with RDE, virus particles are still adsorbed(7), although to a lesser degree. Studies of the elution of myxoviruses from white blood cells have given inconsistent results(7,8,9). In recent work it has been shown that blood platelets also adsorb some myxoviruses and become agglutinated(10, 11). In the present study the adsorption and elution of myxoviruses to and from blood platelets is compared to that occurring with red blood cells.

Materials and methods. Platelets. 18 ml of human venous blood were drawn into 2 ml of a solution of 1% Na_2 sequestrene, 1% triton and 0.7% NaCl. Siliconized (Dow Corning D.C. 200) glassware was used throughout. After centrifugation at 1000 rpm for 15 minutes, the plasma was separated and centrifuged at 4°C for 15 minutes at 2000 rpm. The suspension was then diluted 40 fold with 0.15 M phosphate buffered saline (pH 7.2) and washed twice by centrifugation at 2000 rpm for 15 minutes at 4°C. The final sediment was suspended in buffered saline to a concentration of about 5×10^5 platelets per cmm. *Red blood cells.* The red blood cells obtained after the first centrifugation of the blood sample were washed twice in buffered saline by centrifugation at 4°C at 2000 rpm for 7 minutes. A final concentration in buffered saline of 83.000 cells per cmm was prepared. At these respective concentrations platelets and red cells have approximately equal total cell surfaces; this is required for comparison of kinetics of adsorption and elution between the 2 types of cells. The surface of a red blood cell according to Ponder (12) is 163 μ^2, and the surface of a platelet was calculated to be 28.3 μ^2 (assuming a spheric form and a radius of 1½ μ(13). *Virus.* Influenza A, strain FM_1, and Newcastle Disease Virus (NDV), strain HP(14) were used. Stock virus was prepared by injecting 0.2 ml of 10^{-3} dilutions of infected allantoic fluid into the allantoic sac of 9 days old chick embryos. The infected allantoic fluid was harvested after 48 hours of incubation at 36°C and stored at –20°C. Before use the virus was purified and concentrated by one cycle of adsorption and elution according to Francis and Salk(15). Dialyzed virus was prepared according to Ginsberg and Blackmon(7). The potency of virus suspensions was determined by hemagglutination: 0.2 ml of a 1% suspension of chick red blood cells was added to a series of dilutions of the virus (1:1.5) in 0.2 ml in 0.15 M phosphate buffered saline (pH 7.2) and after suitable incubation the hemagglutination titer was read according to Salk(16). *Receptor destroying enzyme.** Titration of the enzyme was performed in a solution containing 1 g $CaCl_2$, 9 g NaCl, 1.203 g H_3BO_4, 0.052 g $Na_2B_2O_7 \cdot 10H_2O$ in 1000 ml of distilled water. To 0.25 ml of dilution of the enzyme were added 0.25 ml of a 1% suspension of red blood cells. After incubation for 30 minutes at 37°C, 0.2 ml containing 10 hemagglutinating doses of influenza virus per ml was added. After incubation at 4°C for one hour the titer of enzyme was determined as the highest dilution which inhibited the agglutination of red blood cells by virus. For receptor destruction, 10 units of RDE were added to a 1% suspension of red blood cells or to a corresponding quantity of platelets. After incubation for 30 minutes at 37°C the cells were washed by centrifugation at 2000 rpm for 10 minutes. *Electrophoretic mobility.* Electrophoretic measurements(17) were carried out at 25°C in a Northrop-Kunitz type micro-electrophoresis apparatus, equipped with reversible electrodes (copper in saturated copper sulfate) and mounted on the stage of a phase-contrast microscope. All measurements were taken in the lower stationary layer. Measurements for each mo-

* Behringwerke, Marburg-Lahn.

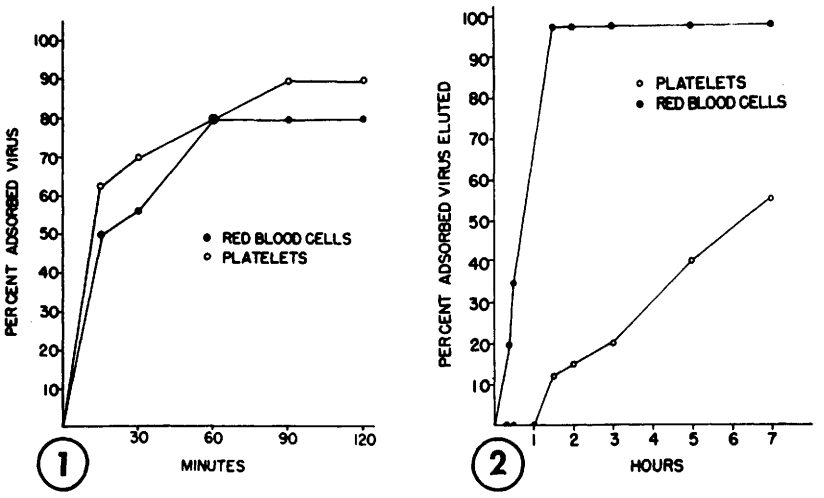

FIG. 1. Adsorption of influenza FM_1 virus to platelets and red blood cells at 4°C.
FIG. 2. Elution of influenza FM_1 virus from platelets and red blood cells at 37°C.

bility determination were performed on 20 platelets, 10 before and 10 after reversal of the direction of the field. *Adsorption and elution of viruses.* To 0.25 aliquots of cell suspension, 0.25 ml of virus suspension was added (hemagglutination titer influenza FM_1-1:1250 and NDV HP-1:700). The tubes were incubated at a desired temperature, and after consecutive time periods centrifuged at 2000 rpm for 10 minutes. The supernatants were titrated for the hemagglutinative titer, and the percentage of virus adsorbed calculated. Following an adsorption period of 90 minutes at 4°C, the cells were spun down, washed, resuspended in saline and then incubated in a 37°C water bath for varying periods of time. After centrifugation, the hemagglutination titer of the supernatants was determined and the percentage of virus eluted calculated.

Results. Adsorption. The time-curve of adsorption of NDV and influenza viruses to blood platelets at 3 temperatures, 4°, 23° and 37°C, resembled that observed for red blood cells. Fig. 1 represents comparative curves of adsorption of influenza virus to red blood cells and to platelets at 4°C. Similar curves were obtained when the reaction proceded at 23° and at 37°C. Adsorption of influenza virus to platelets was maximal at 4°C, while that of NDV was maximal at 37°C, similar to adsorption onto red blood cells.

Cations. The influence of cations on the adsorption was studied in an isotonic glucose-NaCl solution containing varying NaCl concentrations, using dialyzed virus brought to the same ionic strength. Table I shows that adsorption of influenza virus to red blood cells ceases at a concentration of 0.01 M NaCl, while the virus was retained by platelets even when concentration of NaCl was as low as 0.0025 M.

Elution. There was a striking difference in the kinetics of elution of the 2 myxoviruses from the red blood cells on the one hand and from the platelets on the other. Under comparable conditions less virus was eluted from platelets than from red blood cells and the process of elution from platelets took a much longer time. This difference for influenza virus is seen clearly in Fig. 2.

Red blood cells from which influenza virus had been eluted were unable to adsorb additional virus, whereas platelets still adsorbed small quantities of the same virus after a single cycle of adsorption and elution (Table II).

Similar results were obtained when the receptors on red blood cells and on platelets were destroyed by RDE. Following the treatment with this enzyme the red blood cells became incapable of binding influenza

TABLE I. Adsorption of Influenza Virus to Red Blood Cells and to Platelets at Decreasing NaCl Concentration.

	Molar conc. of NaCl				
	.155	.100	.050	.010	.0025
	% of virus adsorbed*				
Platelets, 5 × 10^5/mm³	87	87	87	56	56
Red blood cells, 8.3 × 10^4/mm³	70	70	56	0	0

* % of hemagglutination units of virus which disappeared from the supernatant after adsorption for 90 min. at 4°C.

virus, while the platelets still adsorbed a small amount (Table III).

Treatment of platelets and of red blood cells with RDE, reduced the electrophoretic mobility of both, almost to the same extent (Table IV).

Discussion. The results of the experiments described here indicate that the receptors for myxoviruses on red blood cells and on platelets may be identical. This contention is supported by the similarity of extent and kinetics of adsorption of NDV and influenza virus to both types of blood cells, as well as by the reduction of electrophoretic mobilities of these cells by RDE treatment. In view of receptor identity, the incomplete elution of virus from the platelets as compared to the almost complete elution from the red blood cells may be explained by assuming that virus is incorporated by the platelets. Danon *et al.*(18) demonstrated in electronmicrographs influenza virus particles inside vacuoles of the platelets.

The ability of the platelets to remove virus from the medium in absence of cations, and even after previous adsorption of virus and its elution, or after RDE treatment demonstrates further differences in the interaction

TABLE II. Adsorption of Influenza Virus to Red Blood Cells and Platelets from Which Influenza Virus Had Been Eluted.

	% virus adsorbed	
	Previously unadsorbed	Following elution
Platelets, 5 × 10^5/mm³	80	30
Red blood cells, 8.3 × 10^4/mm³	70	0

of viruses with platelets and red blood cells. This, too, may be explained by uptake of virus particles by the platelets. A similar consideration may be valid for the white blood cell which incorporates virus particles in absence of cations(7) and following RDE treatment (7). Finally, the delay in initiation of elution of influenza and NDV viruses from platelets and its slower rate may be related to morphological and biochemical changes occurring in the platelets at 37°C. At this temperature viscous metamorphosis occurs(19), resulting in loss of the hyalomer and subsequent aggregation of platelets(20). Thus, virus particles trapped in vacuoles situated in the subsurface of the platelets could be gradually released following disintegration of the hyalomer. Later, when compact platelet aggregates are formed, some virus particles trapped in vacuoles inside these aggregates, are not liberated into the supernatant even when the incubation is prolonged. This would explain the incomplete elution of virus from the platelets even after 7 hours of incubation at 37°C.

TABLE III. Adsorption of Influenza Virus to Red Blood Cells and to Platelets Following Treatment with RDE.

	% virus adsorbed	
	Untreated	After RDE treatment
Platelets, 5 × 10^5/mm³	70	20
Red blood cells, 8.3 × 10^4/mm³	60	0

TABLE IV. Effect of RDE Treatment on Electrophoretic Mobility (μ/Sec./V/cm) of Platelets and Red Blood Cells.

	Untreated	RDE treated
Platelets	−1.20	−0.53
Red blood cells	−1.00	−0.55

Summary. 1. Influenza and Newcastle Disease Viruses are adsorbed to red blood cells and to platelets in a similar manner. Evidence is presented indicating that there exists similar virus receptors on the surface of the red blood cells and of the platelets. 2. Virus is adsorbed to platelets in the absence of cations as well as after its previous

elution and after RDE treatment of platelets. Furthermore elution of the virus from platelets is much slower and less complete than that from red blood cells. These phenomena may be due to incorporation of virus particles into the platelets.

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Relationship of Human and Bovine Strains of Myxovirus Para-Influenza 3. (26371)

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The first recognized strain of para-influenza 3 virus was the hemadsorption type 1 (HA-1) virus recovered from children with pneumonia and febrile upper respiratory illness(1). A virus (SF-4) with similar properties of para-influenza 3 virus was recovered from cattle with respiratory disease (shipping fever)(2). The original serological comparison of these human and bovine viruses indicated they were antigenically indistinguishable(3). Subsequent studies by Hamparian and Hilleman, however, showed the viruses could be differentiated by use of serum from intranasally infected guinea pigs (4). The work reported here confirms and extends their findings.

Materials and methods. Virus. The human, HA-1, and bovine, SF-4, strains of the virus used to infect the guinea pigs were grown, respectively, in monkey and bovine kidney tissue cultures. The isolation history and passage of these strains has been reported (1,2). The HA-1 pool had an infectivity of $10^{6.5}$ per ml and the SF-4 pool of 10^{7} per ml. In addition, bovine isolates from different parts of the United States, as well as human isolates from 1955-1959 in Washington, D.C., and from Canada, France, England and Australia, were studied.

Soluble antigens. Preparation of HA-1 and SF-4 soluble antigens is described by Cook, *et al.*(5).

Inoculation of guinea pigs. All guinea pigs were prebled and no hemagglutination inhibition (HI) or complement-fixing (CF) antibodies could be demonstrated with either human or bovine strain antigens. Sera of several animals were also tested for presence of