interaction of influenza virus with blood platelets

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the interaction of myxoviruses with red cells and leucocytes has been the subject of numerous investigations. adsorption and elution of myxoviruses onto and from red blood cells (rbc) is believed to be effected through the interaction of the viruses with the neuraminic acid (the presumed receptor-site) in the mucoprotein of the rbc membrane. however, only a few studies have been reported on the interaction of these viruses with blood platelets. it has been shown recently that the neuraminic acid of human blood platelets is subject to hydrolysis by the neuraminidases (receptor destroying enzyme: rde) of vibrio cholerae and influenza virus.

in the present paper, the results are reported of investigations on the interaction of influenza virus of the pr-8 strain with human and rabbit blood platelets in vitro. human and rabbit red blood cells were used for comparative studies. in vivo studies were also done by infusing virus suspensions into rabbits and measuring the circulating platelet count. the survival of radioactively labeled platelets treated with virus prior to infusion was also determined.

materials and methods

the virus. influenza pr-8 virus was grown in embryonated eggs, as described previously. the infected allantoic fluid was harvested as "crude virus." this was partially purified by a single cycle of adsorption-elution with human group o, rh (+) red cells and will hereafter be referred to as "live" virus. for in vivo studies in rabbits, the virus suspensions were further purified by centrifugation at 145,000 g for one hour at 4 c. inactivated ("dead") virus was prepared by incubating the suspension of "live" virus at 56 c. for 30 minutes.

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The potency of the virus suspensions was titrated by the hemagglutination method, using a 0.5 per cent suspension of human group O, Rh (+) RBC in PBS.

**Preparation of Platelets and RBC Suspensions.** Platelets were separated from the blood by differential centrifugation at 4°C, as follows: Human group O, Rh (+) blood, mixed with a 10 per cent volume of EDTA as anticoagulant (1.5 per cent EDTA in 0.7 per cent sodium chloride), was centrifuged at 1000 r.p.m. (225 g) for 15 minutes. The supernatant platelet-rich plasma was separated and centrifuged at 1000 r.p.m. (225 g) for 10 minutes to eliminate most of the residual white blood cells (WBC) and RBC, and subsequently recentrifuged at 3000 r.p.m. (2000 g) for 20 minutes. The platelet button obtained was washed twice with triton-saline and resuspended in cold PBS in measured quantities to obtain the desired platelet concentration. All platelet counts were done by the direct method using phase contrast microscopy.

Human RBC suspensions were prepared from group O, Rh (+) blood washing the cells 3 times with PBS and resuspending them to a 1 per cent concentration in PBS.

Rabbit blood was collected by intracardiac puncture into a syringe containing a 10 per cent volume of EDTA. Suspensions of rabbit RBC and platelets were prepared by the same method used for human cells.

**Preparation and Assay of Neuraminidase (RDE).** The preparation and concentration of neuraminidase from broth culture filtrates of *Vibrio cholerae* (Strain 4-Z) and measurement of the enzyme activity by a modification of the hemagglutination-inhibition method of Burnet and Stone were done as described by Madoff et al.

**Treatment of RBC and Platelets with RDE.** The PBS-suspensions of platelets or RBC were centrifuged at 3000 r.p.m. (2000 g) for 20 minutes at 4°C, and resuspended in tris-saline buffer containing 0.002 mole NaCl. To 2 ml. of these suspensions, 0.1 to 2 ml. of RDE (titer 1/640) was added. The mixtures were incubated at 37°C for 90 minutes, swirled once after 15 minutes, then centrifuged at 3000 r.p.m. (2000 g) for 20 minutes at room temperature. The cells were then washed twice with tris-saline and resuspended in the original volume of PBS.

**Elution of Dead Virus from Cells by RDE.** RBC or platelets to which dead virus was adsorbed were washed twice with normal saline, then resuspended in tris-saline buffer to the original volume of cell suspension. RDE (0.2 ml.) was added and the mixture incubated at 37°C for 1 hour. The suspension was then centrifuged at 3000 r.p.m. (2000 g) for 20 minutes at room temperature, and the supernatant titrated for virus activity.

**Coating of RBC and Platelets with Macromolecules.** A 10 per cent PVP (Polyvinylpyrrolidone) solution was prepared as follows: To 5 Gm. of Plasdone C, 0.45 Gm. of NaCl was added; the mixture was diluted to 50 ml. with distilled water and the pH adjusted to 7.0 with NaOH. The DAS-Gelatin solution was prepared by mixing 8.5 per cent Gelatin dissolved in 0.15 mole sodium chloride with DAS solution (sodium acetate 0.3 Gm., sodium chloride 0.8 Gm., dextrose 5 Gm. per 100 ml., pH 6.3) to a final Gelatin concentration of 1.4 per cent. Dextran of 75,000 average molecular weight was used as a 6 per cent solution in normal saline. The cells were "coated" by suspending in the above solutions and incubating at 4°C. for 15 to 30 minutes and their capacity for adsorption and elution of virus then measured.
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Fig. 1.—The hemagglutination titer of "live" virus suspensions fell sharply with the addition of platelets (Plts) or red blood cells (RBC) to the suspensions at 4 C. The titer returned to the original level, indicating elution of the virus, when the temperature was increased to 37 C.

Platelet Survival Studies. Outbred rabbits weighing 2.5 to 4.5 Kg. were used in these experiments. The lifespan of platelets was measured using homologous platelets labeled in vivo with P32, as previously described. The P32-labeled platelets were prepared in concentrates suspended in 5 ml. PBS solution to which 2 ml. of live or dead virus suspension in PBS (titer varying from 1:5,120 to 1:10,240) was added. After 30 minutes incubation at 4 C. the platelet button was separated, resuspended in 8 ml. of platelet-poor plasma, and infused into normal recipient animals. Each animal was transfused with 11 to 31 x 10⁶ platelets. Control experiments were done using the same procedure, but with no addition of virus.

RESULTS

1. Adsorption and Elution of Virus onto and from Platelets and RBC (Fig. 1). As indicated in Figure 1, the hemagglutination titer (H. A. titer) of live virus suspensions fell abruptly when human platelets or RBC were added at 4 C., suggesting rapid adsorption of the virus onto both types of cells. After washing twice in cold PBS, the cells were resuspended in PBS and brought to 37 C. At this temperature, live virus eluted almost completely in 5 to 30
Fig. 2.—Comparative study of the adsorptive capacity of human platelets and red blood cells for "live" virus.

minutes. Dead virus was also rapidly adsorbed at 4 C. by both platelets and erythrocytes, but did not elute at 37 C. In subsequent experiments it was shown that treatment with RDE resulted in almost complete elution of the dead virus from both platelets and RBC.

The curves of adsorption and elution of live and dead virus onto and from platelets and RBC were almost identical. The adsorptive capacity of platelet suspensions (final concentration: 1.25-2.2 x 10^3 plts/mm^3) almost equaled that of RBC suspensions (final concentration: 0.25 x 10^3 RBC/mm^3).

2. Comparison of the Adsorptive Capacity of Human Platelets and RBC for Live Virus (Fig. 2). The following experiments were done to investigate whether the amount of virus adsorbed was directly proportional to the number and to the surface area of platelets or RBC present in the suspension. Cell suspensions containing 0.0075 to 1.5 x 10^3/mm^3 platelets or 0.00125 to 0.25 x 10^3/mm^3 RBC, respectively, were used for studies of adsorption of virus. Three virus suspensions of different titers were used. The extent of adsorption of live virus was, in almost all instances, the same for platelets and RBC in these suspensions in which the relationship between platelet number and RBC number was 6:1. If the surface of human platelets is about 28.3 square microns (as calculated from an assumed radius of 1.5 microns and an assumed spheric form for these cells), and the surface of human RBC is 163 square microns as measured by Ponder, the total cellular surface was equal in the suspensions in which platelets were sixfold more concentrated than RBC. These results suggested a relationship between the surface area of both platelets and RBC
and the number of receptors for virus adsorption. The findings in Figure 2 also indicated a direct relationship between cell number and capacity for virus adsorption.

3. Effect of Temperature on the Adsorption of Virus onto Platelets and RBC (Fig. 3). The time-course of adsorption of live virus onto platelets and RBC at three temperatures—4, 20 to 30, and 37 C.—was similar for the two cell types (Fig. 3). After 5 to 15 minutes, live virus was almost completely adsorbed onto both cells at all three temperatures. However, while adsorption was maintained or increased throughout incubation at the lowest temperature (4 C.), at 26 to 37 C. slow elution occurred after 30 to 90 minutes of incubation. In other experiments with dead virus, a similar rate of adsorption for platelets and RBC was observed at the three temperatures, but no elution occurred at any temperature.

4. Platelet Changes during Virus Adsorption and Elution (Fig. 4). After adsorption of live virus at 4 C. for 30 minutes, platelet clumping was prominent, while the platelet shape and size, as observed by phase contrast microscopy, had not changed significantly. During the elution period protracted for 3 hours at 37 C., live virus appeared to be completely eluted from the platelets, but clumping of the platelets remained unchanged. It is possible that minute amounts of virus undetectable by the technic utilized in this study may have remained on the surface of the platelets after elution and accounted for the persistence of clumping. Gross alteration of the platelet surface caused by the virus may have also produced increased stickiness and persistent clumping of
these cells. During the elution period, morphologic damage of the individual platelets in the form of swelling, ballooning and fragmentation could be observed.

With dead virus, identical degrees of clumping occurred during the period of adsorption. When these platelet suspensions were brought to 37 C., morphologic changes of the same type and degree as with live virus were observed, although dead virus did not elute.

In control platelet suspensions subjected to the same technical procedures, but without the addition of virus, these changes were minimal or absent. In the presence of live or dead virus, platelet counts decreased strikingly after the washing procedure following adsorption, while the platelet number in control platelet suspensions remained unchanged. During the 3-hour elution period at 37 C., the platelet number did not drop further in spite of the appearance of morphologic changes. After completion of the 3-hour period at 37 C., the platelet counts in the presence of live or dead virus were respectively 46.7 per cent and 33.3 per cent of the original value (1.5 x 10^9/mm^3), while the platelet count in the control suspensions was 96.7 per cent. The decrease in platelet number in the virus-treated preparations may be related partially to the difficulty in counting clumped platelets, but the impression was that destruction of platelets had occurred to a large extent.

5. **Inability of Virus-Treated Platelets to Adsorb Further Live Virus.** Three suspensions of live virus of different titers were used in these experiments. After the first cycle of adsorption-elution, neither platelets nor RBC adsorbed virus again. The process of adsorption and elution of viruses appeared to destroy the capacity of these cells to adsorb further virus.

6. **Adsorption of Live Virus onto Stored Platelets and RBC.** Human platelets and RBC stored for 24 to 72 hours at 4 C. in PBS could still adsorb live virus as actively as in the fresh state. Moreover, platelets suspended in DAS-Gelatin solution^14 and stored for 6 or 22 days still adsorbed live virus completely.

7. **Influence of RDE on the Capacity of Platelets and RBC to Adsorb Live**
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Virus. Previous studies\(^\text{10}\) have shown that RDE releases neuraminic acid, the presumed viral receptor-site, from intact platelets, and it would be expected that platelet-virus relationships would be altered by RDE in a manner similar to that seen following treatment of erythrocytes with this enzyme.\(^\text{13}\) This was demonstrated in a series of experiments in which various numbers of platelets were exposed to various concentrations of neuraminidase. It was shown that neuraminidase in sufficient quantities could abolish completely the capacity of platelets (as well as of red cells) to adsorb virus. Virus suspensions, which were reduced to hemagglutination titers of 1:10 and 1:0 after incubation in the cold with normal platelets or RBC, retained their original activities of 1:640 and 1:320, respectively, after similar exposure to these cells following their treatment with RDE.

8. Influence of Macromolecules on the Adsorption of Virus by Human and Rabbit Platelets. The presence of dextran (3 to 6 per cent), PVP (10 per cent) and DAS-Gelatin (1.4 per cent) in the suspending medium, did not interfere with the adsorption of live or dead virus to human platelets and RBC at 4 C. These cells, presumably coated with the macromolecules, could still adsorb virus completely since there was no difference in the adsorptive capacity of the coated and uncoated cells. Other experiments with rabbit platelets and RBC gave identical results.

9. Comparison of Adsorptive Capacity of Human and Rabbit Platelets and RBC for Live Virus (Fig. 5). As indicated in Figure 5, the adsorptive capacity of human platelets and RBC was definitely greater than that of rabbit cells. In these experiments, various platelet and RBC suspensions from humans and rabbits with equal concentrations of cells and two virus suspensions showing different titers were used. The extent of the adsorptive capacity for live virus by platelets and RBC from rabbits was, in all instances, smaller than that for the respective human cells. It is possible that this difference was due to the difference in size of the cell surface, resulting in a different number of virus receptors since it is known that, in rabbits, red cells (presumably also platelets) are smaller than in humans.\(^\text{15}\) However, it has been shown that sialic acid of human RBC greatly exceeds that of rabbit RBC both per unit area of cell surface and per unit volume.\(^\text{18}\)

10. Changes in Platelet Counts after Infusion of Virus Suspensions (Fig. 6). The intravenous infusion in rabbits, (New Zealand rabbits, 2–3 Kg. body weight) of 2 ml. suspension of either live or dead influenza virus of titers varying from 1:1280 to 1:10,240 always produced a rapid and sharp decrease in the platelet count which reached its lowest value usually between 15 minutes to 1 hour from the time of infusion. Following the initial drop, the platelet counts recovered partially within 1 to 4 hours. Only after 1 day the platelet counts started to increase progressively, returning to normal within 2 to 3 days. The drop in platelet counts was more striking with more concentrated virus suspensions, and Figure 6 only reports the experiments with suspensions of high titer (1:5120 to 1:10240). In general, the decrease in platelet counts was more pronounced, and the return to normal values was somewhat slower, in the experiments with live virus than in those with dead virus. White cell
counts were also followed in these experiments. These showed depression of the circulating white cells of degrees comparable to the depression in platelet counts. The white cell counts, however, returned to normal more rapidly than the platelet counts and usually within 6 to 24 hours. To demonstrate that the changes in platelets and white cells were produced by the virus and not by the suspending fluid, repeated control experiments were done in which rabbits were injected with 2 ml. of PBS-solution or allantoic fluid. No significant decrease in platelet or white cell counts was observed with these preparations.

It is known that the intravenous infusion of inert particles can produce a temporary drop in platelet counts due to adsorption of these particles onto the platelets with subsequent agglutination.37 The question as to whether influenza virus produced a drop in the circulating platelets by a similar mechanism was investigated. Polystyrene latex particles* of 88 mμ in diameter were used in these experiments. This size closely approximates the size of influenza virus,38 assuming that influenza viruses are of the same spheric shape as the polystyrene latex particles. The number of polystyrene latex particles used in most experiments was $0.352 \times 10^{11}$ or $2.816 \times 10^{11}$ and these were suspended in saline. These numbers approximated the numbers of virus particles in 2 ml. suspensions of titer ranging from 1:1,280 to 1:10,240 as used in the rabbits in

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Fig. 6.—The intravenous infusion in rabbits of virus suspensions produced sharp and persistent drop in the circulating platelet counts. These changes were more remarkable with "live" virus. Platelet numbers reported in chart refer to range of preinfusion platelet levels in the rabbits.

the previous series of experiments. This assumption is based on the fact that 0.5 ml. of 0.5 per cent red cell suspension of normal human blood was used in each tube of the hemagglutination test, and work done by Isaacs demonstrates a relationship of approximately 1 to 1 between the number of virus particles and the number of red blood cells at the 50 per cent hemagglutination end point.
The infusion into the ear vein of rabbits of 2 ml. of polystyrene latex particle suspension did not produce any significant change in the number of the circulating platelets. When, however, the 2 ml. suspension contained more particles \((1.75 \times 10^{12} \text{ or } 3.52 \times 10^{12})\), a moderate but consistent lowering in platelet counts could be observed. A striking decrease in the circulating platelets occurred only when 150 to 1000 times more particles were infused. The lowest platelet counts were observed 1 to 4 hours after the infusion of these concentrated particle suspensions; then the platelet counts started to increase gradually and returned to normal within 3 to 4 days.

It seemed clear from these experiments that latex particles infused intravenously were less efficient than virus particles in producing thrombocytopenia in the rabbits.

11. Survival of Rabbit Platelets Treated with Influenza Virus (Fig. 7). Control survival curves of platelets not treated with virus were obtained in ten experiments. These curves are represented in Figure 7 by the shaded area in the 2 upper charts. This area contains the values of the 10 experiments. The maximum recovery—i.e., the highest percentage of platelet radioactivity found in the circulation after infusion of the labeled platelets—varied from 62 to 95
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per cent (average, 78.5 per cent). The survival time—i.e., the time at which 10 per cent of the value of maximum recovery was reached—was 49 to 82 hours (average, 63.8 hours).

As shown in Figure 7, survival time of platelets previously adsorbed with live virus was 19 to 52 hours (average, 38 hours) and values of maximum recovery ranged from 23 to 54 per cent (average, 38 per cent). The survival time of platelets adsorbed with dead virus varied from 57 to 70 hours (average, 65 hours), and the values of maximum recovery were 49 to 71 per cent (average, 56 per cent).

It was apparent from these results that survival of platelets adsorbed with live virus was more markedly reduced than survival of platelets adsorbed with equal amounts of dead virus. Platelets which had been treated with dead virus showed reduced values of recovery, while the survival time of the remaining platelets was normal. On the other hand, platelets treated with live virus showed sharply reduced values of recovery as well as of survival time.

Platelet counts done in the animals during the survival studies of the virus-treated platelets showed sharp reduction from the preinfusion level. This was more marked after the infusion of platelets treated with live virus than with dead virus. These results may indicate that the circulating platelets of the recipient animals were attacked by virus eluted from the infused labeled platelets either during their survival in the circulation or after destruction.

The greater effectiveness of live virus in reducing platelet viability may be related to the capacity of live virus to elute from the platelets at body temperature, leaving behind greatly damaged platelets.

DISCUSSION

The biological function of neuraminic acid (the presumed virus receptor site) in living cells is still unknown. However, it has been clearly established that virus receptors on the RBC are completely destroyed by RDE from Clostridium welchii, Vibrio cholerae, and Diplococcus pneumoniae. Furthermore, some other agents, including proteolytic enzymes and periodate ions, are known to remove the receptors for myxoviruses from RBC and from tissue cells. Pretreatment with these receptor-destroying agents render the RBC inagglutinable by virus and the intact host cells nonsusceptible to virus infection.

Previous studies have shown that neuraminic acid is released from human platelets by the neuraminidases of influenza virus and V. cholerae. Lu observed that influenza virus could be adsorbed by blood platelets and that in this process the platelets became agglutinated. Furthermore, when virus-agglutinated platelets were treated with fluorescein-labeled virus antisera, the association of the virus with the platelets was visible under the fluorescence microscope. Jerushalmy et al. suggested that the receptors for myxoviruses in RBC and in platelets might be identical in quality. In their experiments, Newcastle disease and influenza viruses affected the clotting and clot-retracting activities of platelets in vitro. In further studies, they showed that mixed agglutination of platelets and RBC could be induced by influenza virus in
vitro, and electronmicrography of this phenomenon showed that virus particles served as a link between the platelets and the RBC. The same authors indicated in their experiments that elution of live influenza virus from blood platelets occurred at a slower rate and was less complete than from RBC. They suggested that influenza virus might not only be adsorbed onto the surface of the platelets, but also incorporated into these cells.

Our results are at variance with these findings. By the use of various concentrations of live influenza virus, we could show that the time curves of adsorption of either live or dead influenza virus onto both platelets and RBC were similar, and that live virus could elute in 5 to 30 minutes at 37 C. as completely from platelets as from RBC. The extent of adsorption of live virus was identical for platelets and RBC when the total surface area of the two cell types in the preparations used was approximately equal. This finding gave further support to the idea that influenza virus is adsorbed onto the surface of the platelets and RBC and that the platelets do not, in addition, incorporate the virus to any great extent within their cellular structure. Although influenza virus particles have been observed by electron microscopy in vacuoles inside of the platelets, it is possible that this phenomenon accounts only for a minimal portion of the adsorptive capacity of these cells for influenza virus. Our results suggest that the virus receptors of platelets and RBC are probably identical in nature and location, and that a similar number of receptors exists in a surface unit of platelets and RBC. However, previous studies in this laboratory have shown that, in man, the concentration of neuraminic acid per $\mu^2$ of platelet surface is approximately eleven-fold greater than that of the erythrocyte. Although this fact would seem to be at variance with the observation noted here of a direct correlation between surface area of platelets and RBC and adsorptive capacity for virus, this finding could be ascribed to the limitation of space for virus adsorption onto the platelets despite the higher potential number of receptor sites on these cells.

In our experiments, dead influenza virus did not elute from the platelets at 37 C. nor from RBC. Treatment with RDE produced prompt elution of the virus from both types of cells, further suggesting that the virus receptors on platelets and RBC are identical.

Since in the process of elution of virus from platelets, the virus receptor is destroyed, it is to be expected that after one cycle of adsorption-elution of live virus, no more virus will again be adsorbed. This is known to occur for RBC. Our results were in agreement with this hypothesis and showed that platelets as well as RBC could not adsorb virus after one cycle of adsorption-elution in the presence of excess virus. Contrasting findings by others, indicating that small quantities of live influenza virus could still be adsorbed onto blood platelets after one cycle of adsorption-elution, suggest to us the possibility that the quantity of virus used in these experiments for the first adsorption may have been insufficient for the complete saturation of the virus receptors of the platelets.

The reduction in platelet number and the gross morphologic alterations of the platelets observed during virus adsorption-elution may indicate that during
this process the platelets are not only agglutinated, but also severely damaged and partially lysed. This severe platelet alteration is confirmed by the finding of a greatly shortened survival in vivo of rabbit platelets when these are treated with influenza virus before reinfusion into the rabbit circulation.

It is known that storage of the platelets for prolonged intervals at 4°C greatly reduces the viability of these cells as expressed by reduction in the ATP content, in clot retracting property and in survival in vivo. Our results showed that the extent of virus adsorption by stored platelets is of the same degree as for fresh platelets. Therefore, it seems that for the adsorption of influenza virus, normal metabolic activity of platelets is not necessary. This probably reflects the fact that the neuraminic acid receptor, once formed, is not dependent on cell metabolism and integrity.

Macromolecules—namely, dextran, PVP and presumably Gelatin—are known to coat the platelet surface. Our results showed, however, that they did not cover the binding sites for live or dead influenza virus. According to recent chemical and electron microscopic studies, the entire surface of myxoviruses is covered by spikes extruding from a coat of lipoprotein at the surface. These spikes are assumed to be involved in the interaction between virus and cells. It is possible that these structures are able to penetrate between macromolecules coating the platelet surface.

We can conclude from our results that the interaction of influenza virus with human blood platelets and RBC is fundamentally the same and that the virus receptors on the platelets and RBC are probably identical in nature and location. It is of interest to observe that, on the other hand, the adsorption of influenza virus by leukocytes seems to occur in a different manner since these cells can adsorb more virus than RBC and only a small portion of adsorbed virus can subsequently be eluted. Phagocytosis seems to be the main mechanism by which leukocytes take up virus particles as demonstrated by electron microscopy, and this phenomenon may represent the main defense mechanism of the body against virus infection since it appears that virus particles can be phagocytized by leukocytes from the surface of RBC.

The studies presented here on the interaction of blood platelets with influenza virus may also contribute to the understanding of the thrombocytopenia which has been repeatedly observed during the acute viremic phase of overwhelming virus infections. The morphologic alterations and lysis of the platelets occurring during virus adsorption and elution in vitro indicate that in case of viremia blood platelets may carry virus in the circulation and be damaged and destroyed in this process.

**Summary**

The interaction of human blood platelets with influenza virus (PR-8) was studied in vitro and in vivo.

It was found that "live" influenza virus was rapidly adsorbed onto human blood platelets at 4°C and completely eluted at 37°C. "Dead" virus was adsorbed at 4°C but not eluted at 37°C unless the platelets were treated with RDE (receptor destroying enzyme). Adorption of virus also occurred at tem-
peratures above 4 C. (from 20 to 37 C.). However, while adsorption was maintained throughout incubation at 4 C., slow elution occurred after 30 to 90 minutes incubation at 26 to 37 C. Storage of the platelets for lengthy intervals at 4 C. or coating of the platelets with macromolecules did not interfere with virus adsorption. After one cycle of adsorption-elution, blood platelets could not adsorb virus again. Treatment with RDE greatly reduced virus adsorption. During the process of virus adsorption, prominent platelet clumping occurred. During elution, clumping remained unchanged, and gross alterations in morphology of the platelets were observed. In the process of virus adsorption-elution, large numbers of platelets were lysed.

Comparative experiments were performed simultaneously with human red blood cells (RBC) and identical results were obtained as with blood platelets. However, the extent of adsorption of live virus was equal for platelets and RBC only when the relationship between platelet number and RBC number in the preparations used was 6:1. This suggested a direct proportion between the surface area of both platelet and RBC and the number of available virus receptors.

Virus suspensions infused into rabbits produced a sharp and sustained drop of the platelet count. Survival of radioactively labeled platelets treated with virus prior to infusion was markedly shortened with live virus and was only slightly reduced with dead virus.

It is suggested from these experiments that blood platelets, as other blood cells, may serve as carriers of viruses in the circulation and that in this process the platelets are damaged and partially destroyed.

**Summary in Interlingua**

Esseva studiate in vitro e in vivo le interaction de thrombocytos human con virus de influenza (PR-8).

Esseva trovate que "vive" virus de influenza esseva adsorbite rapidemente ad thrombocytos human a un temperatura de 4 C e eluite completmente a un temperatura de 37 C. Virus "morte" esseva adsorbite a un temperatura de 4 C sed non esseva eluite a un temperatura de 37 C excepte quando le thrombocytos habeva essite tractate con enzyma a destruction de receptores (EDR). Le adsorption de virus occurreva a temperaturas supra 4 C (ab 20 ad 37 C). Tamen, durante que le adsorption esseva mantenite ab le cominciamento usque al fin del incubation a un temperatura de 4 C, un lente elution occurreva post 30 a 90 minutas de incubation a un temperatura de inter 26 e 37 C. Un preservation prolongate del thrombocytos a un temperatura de 4 C o lor revestimento con macromolecules non interfereva in le adsorption de virus. Post un ciclo de adsorption e elution, le thrombocytos non esseva capace de adsorber virus de novo. Le tractamento con EDR reduceva grandemente le adsorption de virus. Durante le processo del adsorption de virus, un prominente aggregamento thrombocytic occurreva. Durante le elution, le aggregamento remaneva stabile, e grossier alterationes morphologic del thrombocytos esseva observate. In le processo del adsorption e elution de virus, grande numeros de thrombocytos esseva lysicate.

Experimentos comparative esseva interprendite simultaneemente con eryth-
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rocytos human, e le resultatos obtenite esseva identic con illos obtenite con thrombocytos. Tamen, le grado de adsorption de virus vive esseva equal pro thrombocytos e erythrocytos solmente quando le relation inter le numero de thrombocytos e le numero de erythrocytos in le preparatos utilisate esseva 6:1. Isto pareva indicar un proportion directe inter le areas superficial in thrombocytos e erythrocytos e le numeros de disponibile receptores de virus.

Suspensiones de virus infusionate in conihios produceva un marcate e sustentate declino in le numeration thrombocytic. Le longevitate de radioactive-memente marcate thrombocytos tractate ante le infusion con virus esseva marcatemente reducite quando le virus utilisate esseva virus vive sed solo levemente reducite quando le virus utilisate esseva virus morte.

A base de iste experimentos, il es suggestionate que thrombocytos—como altere cellulas del sanguine—pote servir como vectores de virus in le circulation e que in iste processo le thrombocytos es lesionate e partialmente destruite.

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