origin(3). The NRL determination in human urine has been used clinically to evaluate adrenal cortical function(11,12). The administration of ACTH to man causes a considerable increase in the excretion of neutral reducing lipids(13). Burstein(14) has reported that a large increase in excretion of NRL and formaldehydogenic material followed the administration of as little as 100 γ of ACTH to the guinea pig. It was, therefore, startling to observe that even though adrenalectomy reduces NRL excretion to half the control value, doses of ACTH which produce an eosinopenia in the Slonaker-Addis strain of rats do not increase NRL excretion. The failure of anterior pituitary powder to increase NRL excretion confirms the lack of effectiveness of ACTH.

It was even more surprising to observe that an increased NRL excretion was induced by posterior pituitary gland powder. The causative agent in this crude mixture appears to be thermolabile. The substance which increased NRL excretion probably acts through the adrenals, although some increase in excretion does occur in the absence of the adrenals.

Summary. The neutral reducing lipids were determined in the urine of rats. Adrenalectomy decreased the excretion of this material to one-half the control value. Neither ACTH, growth hormone, nor anterior pituitary powder significantly increased NRL excretion. Posterior pituitary powder increased the excretion approximately 3-fold. This response is considerably diminished in adrenalectomized rats.

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Influence of Influenza Hemagglutinin on Hemolysis Rates. (19742)

J. B. BATEMAN, MARY S. DAVIS, AND PATRICIA A. MCCAFFREY.
From Camp Detrick, Frederick, Md.

Much of what is known about the red cell membrane has resulted from observing the effects of various chemical substances upon red cells. Certain of these when added to a cell suspension become attached to the cell surface. This attachment or its sequelae may manifest themselves in many ways, agglutination or lysis being among those most readily observed and least understood.

Although hemagglutinating and hemolysing substances are not always sharply differentiated, there are instances in which the two types of action can be separated quite clearly. In this report, which deals with the successive actions of the two types of substance, the guiding principle has been the idea that effects might be produced which could be interpreted as the result either of simple blockage of one function by the other or of complete independence of the two functions,

this being the simplest working hypothesis that occurred to us. It was realized, of course, that data might be obtained which would compel recognition of more complex interrelationships.

Methods. The choice of hemolytic and hemagglutinating systems was governed in part by considerations irrelevant to the foregoing argument. The PR8 influenza virus agglutinin was used because of an interest in the virus-cell interaction. It is not a typical hemagglutinin, because it produces irreversible effects upon the red cell surface which lead to its spontaneous elution. However, it can be converted by gentle heating into an agglutinin which attaches itself permanently to the red cell. Red cells treated with each of these substances were used in our experiments. The lysin, sodium choleate, was chosen partly for reasons of experimental convenience and partly because it is one of a class of agents that has been much investigated. Useful hypotheses are available concerning their mode of action. A *standard procedure* was used. Washed guinea pig red cells were mixed with the ultracentrifugally purified PR8 virus agglutinin or with the heated virus preparation to give a 1.5% cell suspension by volume, and from time to time the concentration of free agglutinin in the suspending medium and the rate of lysis of the red cells upon addition of sodium choleate were determined. The former was measured by titrating the agglutinin with chick red cells. The latter was determined turbidimetrically, the rate of lysis in the central portion of the sigmoid hemolysis curve being characterized by a unimolecular velocity constant k, the value of which is readily obtained by plotting the logarithm of the percentage of *intact* red cells against time and applying the following equation to the straight line so obtained:

$$2.3 \log (100\text{-}P) = \ln (100\text{-}P) = k\ (t\text{-}a)$$

P is percent hemolysis, t is time in minutes, and a is an intercept obtained by extrapolating the straight line to zero hemolysis.

Results. In nearly all experiments an unequivocal decrease in the constant k was observed during the adsorption phase of the adsorption-elution sequence. This was succeeded during elution by a gradual return to the normal value and then by a significant increase above the normal value. The magnitude of the effects observed can be seen from Table I in which the results of a representative experiment are given in condensed form. In this table the course of adsorption and elution of virus hemagglutinin can be followed by inspecting the column headed T/T_0 in which the amount of free agglutinin is expressed as a fraction of the total agglutinin present in the red cell suspension. The concomitant changes in lysis rate under standard conditions are shown in the final column, in which values less than unity represent inhibition of lysis and values greater than unity represent acceleration. It will be noticed that the process

TABLE I. Effect of Adsorption and Elution of Ultracentrifugally Purified PR-8 Influenza Virus Hemagglutinin on Unimolecular Velocity Constant for Lysis of Guinea Pig Red Cells by Sodium Choleate.

Time, min	T	T/T_0	k_0, min^{-1}	k, min^{-1}	k/k_0
-7	—	—	1.12	—	—
0	512	1	—	—	—
2	—	—	—	.67	.60
4	48	.09	—	—	—
16	—	—	—	.40	.36
17	48	.09	—	—	—
29	—	—	1.12	—	—
43	—	—	—	.62	.55
45	64	.13	—	—	—
54	—	—	1.12	—	—
126	—	—	—	.79	.75
138	—	—	1.05	—	—
178	—	—	—	.80	.78
181	384	.75	—	—	—
305	—	—	—	.70	.74
308	512	1	—	—	—
358	—	—	.92	—	—
363	—	—	—	.92	1
364	512	1	—	—	—
408	—	—	.83	—	—
413	—	—	—	.98	1.18
484	—	—	.79	—	—
491	—	—	—	1.18	1.50
494	512	1	—	—	—

Time scale is in min after addition of hemagglutinin to red cell suspension.

T is titer of free hemagglutinin in mixtures of red cells and agglutinin, determined with chick red cells by Salk method.

k_0 is unimolecular velocity constant for lysis of control suspension of guinea pig red cells by sodium choleate. Final concentrations in reaction mixture: red cells, .75 vol %, sodium choleate, .03%.

k is unimolecular velocity constant for lysis of guinea pig red cells in presence of PR-8 influenza virus hemagglutinin.

of elution and the corresponding change in lysis rate did not occur simultaneously. The first sign of reversal of the initial inhibitory effect was detected some time after elution had become evident, and the lysis rate constant continued to increase after complete elution.

The "nonelutable" heated hemagglutinin or "indicator virus" when used in the form of a solution of comparable titer to that of the original elutable material, although completely and permanently adsorbed by the standard guinea pig red cell suspension, produced only a slight and uncertain inhibition of lysis rate.

Discussion. In considering the interpretation of these results it must be recalled that whereas the lysis of red cells by substances like saponin and sodium choleate is without effect upon the average electrostatic charge density of the cell membrane(1), the adsorption and elution of influenza virus hemagglutinin is accompanied by a very great decrease in charge density(2). The discovery that lysis is inhibited when the agglutinin is adsorbed suggests that the sites of adsorption of lysin are situated somewhere on the virus receptor spots and may be the points of initial attachment of the virus agglutinin, this being followed by a chemical action involving a sufficiently large portion of the surrounding surface to cause a change in the average charge density of the entire surface.

This simple hypothesis of key spot blockade meets with some difficulty when the results obtained with the nonelutable agglutinin are considered. The proportion of the cell surface covered by the nonelutable substance must have been at least as great as the maximum proportion covered during the adsorption-elution of "active" virus, yet the inhibitory effect was slight. In future experiments attention will be given to two alternative hypotheses: (1) that the adsorption of heated agglutinin is less specific than that of the original substance; (2) that the inhibition in presence of the elutable material is caused by a reaction product and not by the agglutinin itself.

The results reported here are at first sight at variance with those of a single experiment described by Briody(3). However, his experiment differs, since he worked with a complex system containing uncontrolled amounts of several extraneous substances.

Summary. 1. The rates of lysis of guinea pig red cells by sodium choleate are altered by the adsorption and spontaneous detachment ("elution") of purified PR-8 influenza virus hemagglutinin. During the adsorption phase, lysis is inhibited; after detachment of the agglutinin, there is a delayed increase in rate of lysis to values above normal. 2. Considered in conjunction with electrophoretic data for red cells treated with virus hemagglutinin, these results suggest that the hemagglutinin first attaches itself to a lytic "key-spot". It then brings about, over a considerable neighboring area of the red cell surface, the changes which are manifested in alteration of the average electric charge density of the surface.

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