there was a significant increase in percentage of polyunsaturated fatty acids in the serum cholesterol ester and triglyceride fractions and the serum cholesterol level was significantly lowered. However, the same shift in dietary regimens did not alter the serum lipid fatty acid spectrum of subjects with initially normal blood cholesterol levels, but produced a significant reduction in serum cholesterol level. When the latter group of subjects was transferred from the high linoleic acid diet to a diet low in that acid there was a significant decrease in percentage of linoleic acid in the serum cholesterol ester and triglyceride fractions, and a significant elevation of the blood cholesterol level. Other factors could account for the difference in varying response to the dietary regimens. One factor could be the difference in the ages of the subjects. The data also suggest that linoleic acid affects the serum cholesterol level independently of its effect on the serum lipid fatty acid spectrum, *e.g.*, when serum cholesterol levels of both groups were the same, the percentage of linoleic acid in the serum cholesterol esters of Group I subjects was lower than in Group II subjects. These findings stress the need for further investigation of the role of essential fatty acids in cholesterol ester transport and metabolism.

Summary. The findings suggest that the level of linoleic acid in the diet, age, and possibly other factors may influence the serum cholesterol level and fatty acid composition of the serum lipid fractions. The significance of these findings is discussed.

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Reaction of Polyoma and Influenza Viruses with Receptors of Erythrocytes and Host-Cells.* (27307)

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Recently, polyoma virus was found to agglutinate RBC (red blood cells) as do the myxoviruses through interaction with mucoprotein receptors. RBC treated with RDE (receptor destroying enzyme) or influenza virus were inagglutinable by polyoma virus. However, the latter was devoid of neuraminidase(1). The present report concerns the relationships between RBC receptors for polyoma and influenza viruses, and since the primary interaction of myxoviruses with RBC has proved to be a useful model of the virus host-cell reaction, experiments were designed to test whether receptors of the mucoprotein type also function in the initiation of infection of host-cells by polyoma virus.

Materials and methods. Viruses. The SE

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polyoma virus (strain 105) was kindly supplied by Dr. S. E. Stewart and has undergone one passage in Swiss mice and 5 in tissue cultures of primary mouse embryo cells. The immediate source of virus was infectious tissue culture fluid (ITCF). Concentrated polyoma virus, used in certain experiments, was prepared from ITCF by centrifugation at 105,000 $\times$ *g* for 2 hours in the Spinco Model L Centrifuge. Two strains of influenza type A virus were used, the NWS-a strain adapted in this laboratory to growth in Ehrlich ascites tumor cells(2) and the PR-8 strain.

Cell cultures. Primary mouse embryo (PME) cells were prepared from mouse embryos in the 14th to 18th day of gestation according to the method of Youngner(3) as modified by Eddy(4). PME cells were grown in 2 oz. prescription bottles seeded with 1 $\times$ 10^6 cells in 5.0 ml of 0.5% lactalbumin hydrolysate (LH) and 10% calf serum in Hanks' balanced salt sloution (BSS). For cell maintenance and viral growth, 2 media were used: for polyoma virus 0.5% LH and 1.5% heat inactivated horse serum; for influenza virus, medium 199(5).

Receptor destroying enzyme (RDE). Partially pure RDE was prepared according to the method described by Ada and French (6). The activity of enzyme preparations obtained was approximately 1280 units per ml when tested against 4 hemagglutinating units of polyoma virus.

Hemagglutination test (HA). Guinea pig red blood cells obtained by cardiac puncture were stored at 4°C in Alsever's solution, washed in phosphate buffered saline (PBS) prior to use, and adjusted to 0.4%. Direct count by hemacytometer indicated standard 0.4% RBC suspensions contain approximately 3.6 $\times$ 10^7 cells per ml. To serial 2-fold dilutions of 0.4 ml of virus in PBS, 0.4 ml of 0.4% red cells were added, bringing the total volume to 0.8 ml. The red cells were allowed to settle at 4°C overnight and read by a pattern method in which the degree of agglutination produced by limiting 2-fold dilutions was read and by estimating the concentration between dilutions which produced complete agglutination.

TABLE I. Modification of RBC Receptors by RDE.

	Effective RDE dilution[a]			
	Crude filtrate		Purified RDE	
Virus	Heated[b]	Unheated	Heated[b]	Unheated
Polyoma	<2	32	<2	256
Influenza	<2	8	<2	32

a. Reciprocal of highest dilution of receptor destroying enzyme (RDE) preventing hemagglutination.
b. 56°C, 30 min.

Experiments and results. Modification of HA reaction by treatment of cells. To 0.2 ml of 2-fold serial dilutions of RDE in saline, 0.4 ml of RBC at a concentration of 0.4% in saline was added and incubated 30 minutes at 37°C. After incubation 0.4 ml of saline containing 4 HA units of influenza or polyoma viruses was added, mixed and allowed to settle at 4°C. RBC treated with certain concentrations of RDE (dilutions of 1 to 8 or less of crude enzyme and 1 to 32 or less of purified enzyme) were rendered inagglutinable to either influenza or polyoma viruses(1) (Table I). However, smaller concentrations (corresponding dilution of 1 to 32 or 1 to 256) altered them so as to be inagglutinable by polyoma virus but yet agglutinable by influenza virus (Table I). The receptors of the two viruses, while being of the same chemical nature, can be seen also to differ.

Similarly, the effect of potassium periodate (KIO_4) on the agglutinability of RBC by these viruses was determined by mixing equal volumes of 5.0% RBC and varying concentrations of KIO_4 and incubating at 4°C for one hour. Excess KIO_4 was neutralized by addition of an equal volume of 5.0% glucose. The cells were then washed by 4 cycles of centrifugation at 1500 rpm for 10 minutes and resuspensions in PBS containing 1% glucose. The treated RBC were then resuspended in PBS at a concentration of 0.4% and their agglutinability by polyoma and influenza virus (PR-8) determined as described for the HA reaction. Guinea pig RBC critically treated with KIO_4 (Table II) are agglutinated by influenza virus (PR-8) only at 4°C, mimicking the conditions required by polyoma virus. The viral reactivity of such cells is also reduced, since more virus is re-

TABLE II. Modification of RBC Receptor by Potassium Periodate.

Concentration of KIO_4	HA titer[a] – Polyoma virus		HA titer – △[b] influenza virus	
	4°C	22°C	4°C	22°C
M/2000	< 2	< 2	4	< 2
M/3000	2	< 2	8	4
M/4000	4	< 2	8	6
Untreated	8	< 2	12	12

a. Reciprocal of highest dilution producing hemagglutination at either 4° or 22°C.
b. △—heated, 56°C for 30 min.

quired now for their agglutination. With either reagent it is possible to alter the hemagglutination reaction selectively and in both instances the RBC receptors appear more sensitive when tested with polyoma virus. Alternatively, if there are distinct receptors for both viruses of equal sensitivity, then there must be fewer receptors for polyoma virus. The ability to alter the nature of the HA reactions of these viruses by modifying the RBC surface does not necessarily imply that the nature of the HA reaction is a property only of the RBC surface. The following experiments bear on this.

Modification of HA reaction by treatment of influenza virus. The neuraminidase activity of influenza virus (PR-8) was inactivated by dialyzing infectious allantoic fluid against 0.1 M sodium citrate and heating aliquots of the dialyzed virus at 56°C and 65°C for 30 and 60 minutes, respectively. An aliquot of virus heated at 65°C for 60 minutes was removed and heated an additional 60 minutes. The HA characteristics of the various preparations of heated influenza virus are shown in Table III.

TABLE III. Hemagglutination Reactions of Various Heated Influenza Virus Preparations.

Treatment[a]		HA[b] titer	
°C	Min.	4°C	22°C
56	30	1600	1600
65	60	256	64
65	60		
Followed by		32	4
66	60		

a. Preheating of the virus at various degrees centigrade for various times.
b. Reciprocal of highest dilution producing hemagglutination tested at 4° or 22°C.

The HA titer of influenza virus heated one hour at 65°C or after an additional hour at 66°C was 4- to 8-fold higher at 4°C than at 22°C, while the titer of virus heated at 56°C for 30 minutes was the same at both temperatures.

Hemagglutination produced by dilutions of critically heated (66°C; one hour) influenza at 4°C is unstable at room temperature, but will reagglutinate when brought back to 4°C. Thus it is possible to modify the HA reaction by treatment of the virus with heat in such a way that it resembles results obtained by treatment of RBC with the periodate ion. The HA produced at 22°C with undiluted preparations of this virus is probably due to residual contaminating virus not converted by the heating. Thus a particular quality of HA reaction is produced by modifying either the surface of the RBC or the virus.

RBC saturation values for polyoma and influenza viruses and their reciprocal exclusion. Adsorption values of RBC for polyoma and heated (56°C; 30 minutes) influenza viruses were determined by exposing 0.5 ml of 0.5% RBC to 4.5 ml of varying virus concentrations for 4 hours at 4°C. Virus treated RBC were sedimented and washed twice with cold PBS at 4°C. The quantity of adsorbed hemagglutinin was determined as the difference between the original virus concentration and that recovered in the supernatant and washings. These adsorbed quantities of polyoma and heated influenza virus are plotted in Fig. 1 against the virus concentrations to which the cells were exposed. The data indicate that adsorption values are a function of the virus concentration used since even with low concentrations, virus uptake is never complete. Adsorption values for polyoma virus are always less than those for influenza and with increasing concentrations of virus the adsorption curves become increasingly divergent. Saturation of RBC with polyoma virus occurs after exposure to a concentration of approximately 450 hemagglutinating units (HAU) per 0.4 ml, while maximum adsorption of influenza virus does not appear to be obtained with the concentrations used.

Because of similarities of receptors for polyoma and influenza virus(1), experiments

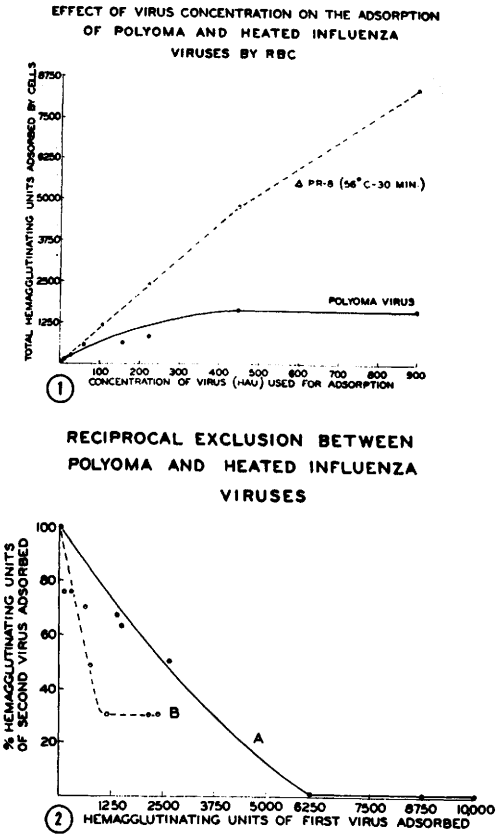

FIG. 1 and 2

In Fig. 2, Curve A represents adsorption of polyoma virus by 0.5 ml of 0.5% RBC after adsorption of varying amounts of Δ-PR-8 virus and is expressed as % of adsorption to untreated cells. Curve B represents adsorption of Δ-PR-8 virus by 0.5 ml of 0.5% RBC after adsorption of varying amounts of polyoma virus and is expressed as % of adsorption to untreated cells.

were designed to test the capacity of each to exclude the adsorption of the other. RBC exposed to various concentrations of virus, as described in the preceding experiment, were tested for their ability to adsorb influenza virus if the first adsorption was with polyoma virus or polyoma virus if the first adsorption was with influenza virus. The results (Fig. 2) indicate that complete exclusion of one virus by prior adsorption of the other is not reciprocal. The prior adsorption of increasing concentrations of influenza virus reduces the capacity of RBC to adsorb polyoma virus and the adsorption of 6062 HAU or more of influenza virus will completely exclude the adsorption of any detectable polyoma hemagglutinin. Adsorption of increasing concentrations of polyoma virus onto RBC also reduces the amount of influenza virus that can be adsorbed. However, maximum adsorption to RBC of polyoma virus does not completely exclude the uptake of influenza hemagglutinin.

Effect of RDE on host cell receptors for polyoma virus. Since preliminary experiments indicated that prior treatment of PME cells with RDE did not materially affect the uptake of polyoma hemagglutinin, the following experiment was designed to test the state of infection of such cells. Monolayer cultures containing approximately 1.5×10^6 cells were pre-treated with 1280 units of RDE prior to infection with 4 HAU of either polyoma or influenza virus. Because it is difficult to remove all the unadsorbed inoculum and it is known that mucoprotein receptors, once destroyed, may be regenerated by cells in time, the development of polyoma virus was followed in cultures pre-treated with RDE and containing RDE (1280 units) during the entire growth period. Further, uninfected replicate cultures so treated with RDE were tested for the presence of mucoprotein receptors by attempting to infect with influenza virus at the time the first new polyoma virus appeared in the test cultures. In these experiments the titer of polyoma virus HA was 4 on the sixth day. The 6-day-old control cultures and those uninfected but pre-treated and incubated 6 days with RDE were exposed to influenza virus. By 52 hours the HA titer of control cultures was 8 and rose to 16 by 76 hours. Treated control cultures produced a partial hemagglutination reaction at a dilution of 1 to 2 and failed to increase (Table IV). Thus under these conditions the receptors, required for influenza virus, could be demonstrated to be inoperable at both the beginning of the experiment following pre-treatment, as well as at the end of the incubation period when replication of polyoma virus had been completed.

Discussion. Completely reversible viral hemagglutination (stable at 4°C but dissociable at 22°C) is characteristic of polyoma and certain other viruses. They are distinct

TABLE IV. Production of Polyoma Virus in PME Cells Continuously Exposed to RDE and Production of Influenza Virus in PME Cells Exposed to RDE Continuously for 6 Days Prior to Infection.

Treatment	Polyoma HA titer[a] Days 0	4	6	8	Influenza HA titer[a] Hr 0	12	24	52	76
Untreated	<2	2	4	16	<2	<2	2	8	16
Treated with RDE	<2	<2	2	8	<2	<2	<2	<2	<2

a. Reciprocal of highest dilution producing hemagglutination at 4°C.

from myxoviruses whose hemagglutination will dissociate at higher temperatures but irreversibly. Gentle heating of the latter will cause them to combine with RBC irreversibly (7), while more severe heating produces a modification which allows reversible dissociation as with polyoma virus (Table III). Untreated influenza will react similarly when RBC critically modified by KIO_4 are used (Table II). The properties of a virus and those of the cell receptor, together, determine the character of the HA reaction. Viruses may behave differently with the same RBC because of differences in the viral reactive groups which participate in the hemagglutination reaction. But it is also possible that less specific properties of the whole virus particle may, by steric or electric influences, select the kind of cell receptor which is to participate in the reaction. Thus viruses differing in the latter properties may produce distinctly different hemagglutination reactions which involve essentially similar viral reactive groups. Since these properties of one virus may be distinct, hemagglutination reactions may differ on multiple bases. The data presented here are suggestive but do not allow clear interpretations at this level of analysis.

The ability of the viruses to produce mutual exclusion (Fig. 2) and for influenza to completely destroy receptors for polyoma virus(1), indicates that they react with certain common receptors. The inability of polyoma virus to exclude influenza virus completely (Fig. 2) shows some receptors to be in the exclusive domain of the latter. On this basis one may propose 2 classes of receptor, one (IP) which can react with either virus and a second (I) which reacts with influenza but not polyoma virus. Each contains an essential component which is a mucoprotein since severe treatment with neuraminidase or periodate will destroy both.

This model is consistent with the large differences in the saturation numbers of the viruses (*i.e.*, the ratio of amounts of virus required just to produce agglutination of a standard number of cells to the maximum which can be absorbed). The data suggest that the number of receptors which can react with each is different and that there are clearly more reactive sites for influenza than polyoma virus. However, there is no agreement among published data(8,9,10,11,12) as to the number of virus particles required for minimal agglutination with either virus. Hence the absolute values of the saturation numbers are of limited use in calculating the absolute number of cell receptors.

The basis of the differing HA reactions of these viruses might be assigned either to the quality of their reactive groups or to the nature of the receptor involved, if the temperature stability of the complexes they form at the IP receptor could be compared. Unfortunately observation of the influenza complex is confused by the presence of the I receptor. However, with normal or progressively modified RBC, polyoma virus always forms less stable complexes than influenza virus. Progressive modification of receptors with 2 reagents (RDE and KIO_4) which should have minimal selectivity for receptor types, results in a more rapid loss of the ability to react with polyoma (Tables I and II). This could reflect only the paucity of IP receptors which fall below a critical level first, except that the behavior of influenza virus indicates a change in the quality of its receptors. Since in each circumstance the viruses act differently and polyoma is always less reactive, it seems

likely that the reactive groups on the viruses also differ in quality.

Knowledge of the reactive chemical moiety of the RBC which the viruses share contributes little to understanding the infection of host-cells by polyoma virus. Here the RBC is not a useful model. Host-cell receptors of this agent appear not to be mucoproteins, are distinct from those of influenza virus, and presumably operate at 37°C where the RBC receptor complex is unstable. Analogous to polyoma receptors of RBC and host-cells are 2 proteinaceous bovine serum inhibitors. One reversibly (at 4° and 22°) blocks the HA reaction without neutralizing infectivity(13). A second acts at 22° to block both infection and the HA reaction(14). It is anticipated that these substances upon isolation will be valuable for further study of the reactive surface of polyoma virus.

Summary. Evidence is presented for the existence of 2 types of erythrocyte receptors for the PR-8 strain of influenza virus; one of these is available also to polyoma virus. Modifications of the quality of the hemagglutination reaction, resulting from treatment of either virus or red cell, are described. While a similarity in the receptors for influenza virus on the host-cell and the erythrocyte can be demonstrated, these were found to be distinct in the case of polyoma virus.

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Effect of RNA and Yeast Autolysate on Experimental Infection in Irradiated and Unirradiated Mice.* (27308)

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Detre and Finch(1) found a marked reduction in mortality (30 days) from a lethal dose of X-radiation in mice injected intraperitoneally or intravenously with yeast ribonucleic acid (RNA) or yeast autolysate 30 minutes before exposure. The following experiments were undertaken to determine whether this protective effect might have been due, in part at least, to increased resistance to bacterial infection, since the development of endogenous infection plays a significant role in the death of mice exposed to mid-lethal doses of ionizing radiation(2). Mice were accordingly treated with RNA or yeast autolysate 24 hours or 30 minutes before, or on the fourth day after irradiation (475 r) and challenged on the fifth day post-irradiation by intraperitoneal inoculation with *Pseudomonas aeruginosa.*

Materials and methods. Mice. CF-1 females, 10 weeks old, were housed in stainless

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