Hemolysis of Human Red Cells by Saponin Following Viral Action[1]

B. A. Briody[2]

Walter and Eliza Hall Institute, Melbourne

Changes in human red cells, including the removal of virus receptors and the development of "panagglutinability," are produced by the action of viruses of the influenza group (*2*). When these altered human erythrocytes were subjected to hemolysis by saponin, it became apparent that another modification was produced as a result of viral action.

Shope's strain 15 of swine influenza virus (SW) was selected to alter the cells, since it removes the virus receptors for most of the other strains of influenza virus (*2*). In order to minimize the time that the human red cells would have to be placed at 37° C, the virus receptors were not removed by the elution technic of Hirst (*4*) but by the following method: Allantoic fluid of the chick embryo infected with SW was added to packed human red cells of Group O and the mixture placed in a water bath at 37° C for 2 hrs. The cells with the adsorbed virus were washed with saline, and, after removal of the supernate, a sufficient quantity of anti-SW ferret serum was added and the mixture placed at room temperature for 15 min. The cells were washed twice with 10 times their volume of saline and then made up to a 4% suspension in saline. The virus receptors were now removed from the human cells. In the lytic experiments to be described, two types of controls were employed: (1) cells treated similarly with uninfected allantoic fluid, and (2) cells whose receptors were coated with SW.

TABLE 1

Hemolysis of Virus-treated Human Red Cells by 1:15,000 Saponin

Treatment of cells	Time room temp. (min)	Photoelectric colorimeter reading			50% hemolysis
Virus receptors intact	10	0.086	0.087	0.092	0.098
	20	0.165	0.163	0.170	
	30	0.229	0.228	0.230	
Virus receptors coated	10	0.083	0.080	0.080	0.105
	20	0.150	0.168	0.168	
	30	0.223	0.235	0.232	
Virus receptors removed	10	0.106	0.106	0.107	0.122
	20	0.117	0.121	0.119	
	30	0.126	0.133	0.128	

The hemolysis resulting from the addition of 0.5 ml of a 1:15,000 dilution of saponin to 0.5 ml of a 4% suspension of the treated human red cells was determined by means of a photoelectric colorimeter. The results recorded in Table 1 show that cells whose virus receptors have been removed are significantly more resistant to hemolysis by saponin than are cells whose receptors are intact or cells whose receptors are coated by virus. There is no significant difference between the latter.

In view of the essential similarity in the action of filtrates of the 4Z strain of *Vibrio cholera* in removing virus receptors from human red cells, the effect of saponin on cells treated with the cholera filtrates was tested. The virus receptors were removed by adding the cholera filtrate in borate buffer at pH 7.6 containing 0.1% calcium chloride to packed human cells at 37° C for 2 hrs. The cells were washed twice with 10 times their volume of saline and made up to a 4% suspension in saline. The virus receptors having been thus removed, the cells were placed in contact with saponin and the degree of hemolysis determined as above, except that a 1:18,000 dilution of saponin was used. The results recorded in Table 2 show that cells whose virus receptors are removed by the cholera filtrate are significantly more resistant to hemolysis by saponin than are cells whose virus receptors are intact. The cells with unaltered virus receptors reached the 50% hemolytic end-point in 20 min, whereas cells without virus receptors did not reach the same end-point until the saponin had acted on the cells for 50 min.

TABLE 2

Hemolysis of Cholera Filtrate-treated Human Red Cells by 1:18,000 Saponin

Treatment of cells	Time room temp. (min)	Photoelectric colorimeter reading			50% hemolysis
Virus receptors intact	10	0.088	0.087	0.087	0.110
	20	0.111	0.105	0.109	
	30	0.130	0.125	0.129	
	50	0.161	0.155	0.162	
	60	0.170	0.164	0.173	
Virus receptors removed	10	0.075	0.074	0.079	0.112
	20	0.084	0.082	0.087	
	30	0.096	0.092	0.095	
	50	0.113	0.113	0.118	
	60	0.123	0.120	0.127	

Cells whose virus receptors are removed either by viral action or by the action of cholera filtrate are not modified in their susceptibility or resistance to hemolysis by hypotonic solutions. Preliminary results indicate that trypsin in a dilution of 1:100 hemolyzes cells whose receptors have been removed more readily than cells whose receptors are intact. The fact that both virus and cholera filtrate alter the resistance of human cells to hemolysis by saponin is in accord with other evidence which suggests that the modifications are induced by an enzyme common to the virus and the cholera filtrate (*1, 3, 6*).

The mechanism of hemolysis is unknown. The most generally accepted theory holds that hemolysis involves the process of penetration and breakdown of a mixed protein-lipoid film at the cell surface (*5*). Since the removal of virus receptors impairs hemolysis by saponin,

[1] Aided by a grant from the National Foundation for Infantile Paralysis.

[2] Fellow in the Medical Sciences of the National Research Council. Present address: The School of Public Health, University of Michigan.

the component of the red cell affected by saponin might be identical with the virus receptor. However, the action of the virus and the cholera filtrate might make more accessible an inhibitor of saponin, such as a protein or lipid.

References

1. BRIODY, B. A. *J. Immunol.*, in press.
2. BURNET, F. M., MCCREA, J. F., and STONE, J. D. *Brit. J. exp. Path.*, 1946, **27**, 228.
3. BURNET, F. M., and STONE, J. D. *Aust. J. exp. Biol. med. Sci.*, in press.
4. HIRST, G. K. *J. exp. Med.*, 1942, **76**, 195.
5. PONDER, E. *J. gen. Physiol.*, 1946, **29**, 203.
6. STONE, J. D. *Aust. J. exp. Biol. med. Sci.*, 1947, **25**, 137.

IN THE LABORATORY

Photograms of Small Bones and Skulls

ERNEST C. DRIVER

Department of Zoology, Smith College

In work involving the comparison of small skulls and bones, especially those prepared by the alizarine-potash method, the writer found the usual photographic methods inadequate and time consuming.

Pictures with good depth of field and with definite and exact degree of enlargement were quickly and easily made by the use of a simple wooden frame in a regular condenser-equipped enlarger. Bolts were removed to separate the enlarger at the place where the negative carrier is inserted, and a black-painted wooden frame, high enough to accommodate a standard finger bowl, was fastened in place of the negative carrier. Fig. 1 shows the general

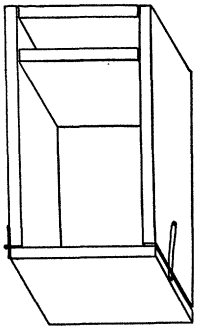

FIG. 1

plan of construction and bracing. A piece of clear glass, such as a cleaned photographic plate, was placed below it to support the specimen in its container and to avoid the possibility of spilling fluid on bellows or lens.

In use, the specimen, in a finger bowl or other suitable container partly filled with glycerine, is placed in the frame and the image focused as in ordinary enlarging. If a fair depth of glycerine is used, the specimen may be kept far enough from the bottom of the dish so that any irregularities in the latter will not be superimposed on the image. The specimen may be oriented through the opening made by the hinged part of the frame. A piece of transparent celluloid ruler, placed in the same plane in the glycerine as the specimen, will also be projected and will give the operator a ready method of determining (and recording on the margin of the print, if desired) the degree of enlargement. When orientation and degree of enlargement are satisfactory, the front part of the frame is closed and held by a rubber band, to avoid stray light, the lens stopped down to f. 16, and the exposure made on regular, normal-grade, enlarging paper. Development will result, naturally, in a white image on a black background. The thinner parts of the bones and sutures allow some light to pass, so that the resulting print, if exposed and developed for detail rather than for contrast, will have an appearance similar to that of an X-ray.

The writer and his students have found this to be a rapid and easy method, giving results much superior to those obtainable with the cameras at their disposal.

Apparatus for Partition Chromatography on Paper

F. C. STEWARD, W. STEPKA,[1] and JOHN F. THOMPSON[2]

Department of Botany, University of Rochester

Increasing use of the methods of partition chromatography on paper (*1*) in the separation and detection of amino acids, peptides (*2–5, 12, 13*), organic acids (*11*), and sugars (*8, 9*), and the obvious applications of the method in conjunction with radioautographs (where the compounds separated by the chromatogram contain radioactive isotopes, *6, 7*), arouse interest in devices which enable the technique to be conveniently performed. A recent note by Longenecker (*10*) describes a method of making the glass troughs from which the solvents are made to flow over the paper in the manner familiar to users of this technique. In making the technique suitable for routine and quantitative work we have experimented in this laboratory with various types of troughs. The note by Longenecker prompts us to describe our solution of this part of the problem in ad-

[1] Now of the Division of Plant Nutrition, University of California.

[2] Fellow of the National Institute of Health.