creased syneresis during mitosis, and (c) decreased cytoplasmic bubbling during mitosis. 3. With a similar beam current, intermitotic cells show (a) cessation of pinocytosis, and (b) cessation of Brownian motion of cytoplasmic lipid droplets, even though no change occurs in the absorption image or the structural image of the cell. 4. When the beam current is further increased, intermitotic cells show a sudden generalized increase in absorption followed immediately by a marked contraction of the cell into a small round intensely absorbing mass.

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A Stable Preparation of Antigen-Sensitized Erythrocytes. (23302)

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In work reported elsewhere(1), the hemagglutination technic devised by Boyden(2) was used to assay the antibody-forming capacity of spleen cultures from rabbits given either bovine serum albumin (BSA) or bovine γ-globulin (BGG). This method, however, has the disadvantage of not making a stable preparation of antigen-sensitized erythrocytes, requiring preparation of freshly sensitized cells for each day's titrations. The present paper describes a method by which sensitized cells may be prepared which remain stable in the frozen or lyophilized state.

Methods. A 50% saline suspension of washed sheep red blood cells was treated with an equal volume of a 37% formaldehyde solution containing 0.9% NaCl for 2-3 days at 4°C. Since formaldehyde causes clumping of the cells, it was usually necessary to disperse these clumps by homogenization in a Waring blendor for 1 hour at 2-5°C. After centrifuging, only the heavy cell layer was saved. No attempt was made to save the lighter material which separates to the surface as a pellet. The formaldehyde was removed by 7-8 washings in 5-6 volumes of saline over a ten-day period, followed by treatment of a 25% cell suspension with 5% $NaHSO_3$ at 4°C overnight. The formaldehyde-bisulfite complex was then removed by dialysis against running tap water for 24 hours. Eegriwe's chromotropic acid test for formaldehyde(3) was used to detect residual formaldehyde. Unless all formaldehyde is removed, spontaneous agglutination occurs. A 50% suspension of the formalinized cells in saline was stable at 4°C for at least 5 months. The sensitization of the cells to the protein antigens after formalinization was essentially the same as the Boyden technic(2). However, when several liters of cells were prepared at one time, the times of treatment with tannic acid and antigens were extended to 30 minutes and one hour respectively. The sensitization was carried out as follows. One volume of a 2.5% suspension of formalinized cells was incubated at 37°C for 30 minutes with 1 volume of a 1:20,000 dilution of tannic acid (Merck, reagent grade) in 0.9% NaCl. The cells were washed once with 0.15 M phosphate buffered saline (PBS) at pH 7.3, centrifuged at 1500 rpm for 5 minutes and resuspended to volume in saline. One volume of these cells was incubated at room temperature for 1 hour

TABLE I. Hemagglutinin (HA) Titers of Anti-BGG Culture Fluids Using Normal Freshly Sensitized Cells, Formalinized Frozen Sensitized Cells, and the Latter Lyophilized.

Fluid No.	HA titer		
	Normal	Formalinized	Lyophilized
1	8	16	16
2	8	4	8
3	4	8	16
4	8	8	8
5	16	32	64
6	16	8	8
7	8	16	16
8	32	32	64
9	16	32	64
10	8	32	32
11	32	64	64
12	64	128	128
13	16	32	32
14	8	8	8
Anti-BGG serum	12,800	25,600	12,800
Idem	25,600	12,800	ND*
"	25,600	25,600	ND
Normal rabbit serum	< 2	< 2	< 2

* Not done.

in 4 volumes of PBS, pH 6.4, containing 0.5 mg/ml of BGG (Armour's Fraction II) or the same amount of BSA (Armour's Fraction V) for each ml of cells to be sensitized. The cells were washed once with 1.0% normal rabbit serum (NRS) in 0.9% saline, centrifuged at 1500 rpm for 10 minutes and reconstituted to a 2.5% suspension in 1.0% NRS in 0.9% saline. These sensitized cells were then frozen at −70°C and stored at −40°C. Microscopically, the cells appear fairly normal and are impervious to hemolysis in hypotonic solutions. The cells are equally stable in the lyophilized state.

Results. Table I shows the results of titrating several tissue culture fluids from spleen cultures of rabbits given BGG intravenously(1). These were chosen at random from fluids frozen 6-8 weeks. A hyperimmune anti-BGG rabbit serum was included for comparison of titers. All titrations were carried out in serial 2-fold dilutions with 1:100 NRS in 0.9% NaCl as the diluent. Fifteen hundredths (0.15) ml of a 2.5% suspension of sensitized cells (about 6 x 10^{10} cells) was added to each tube, the racks shaken and left at room temperature for 2 hours. They were then placed in the cold room and the HA end-points read the following morning. It is seen that the titers are within the range of dilution error (2 tubes) using freshly sensitized normal cells, frozen formalinized cells stored at −40°C up to 5 months, or the latter lyophilized and stored at room temperature for 6 months. Cells sensitized to casein or BSA and titrated with the homologous antiserum gave equally satisfactory results.

Discussion. Flick(4) treated human erythrocytes (group not specified) with formalin, and thus prepared a stable indicator useful for influenza A virus hemagglutination. Since these cells were unadaptable to the Salk pattern method(5) due to spontaneous agglutination, the turbidometric technic(6) was used. Cole and Farrell(7) described a method for coupling tuberculin PPD to formalinized Group A human red blood cells using tetrazotized benzidine. They then were able to titrate serum antibody to tuberculoprotein by an hemagglutination end-point pattern. Two attempts were made in this laboratory to couple BSA and BGG to sheep erythrocytes by this method, but spontaneous agglutination occurred both times. This may have been due to incomplete removal of the formaldehyde, or to a difference in the receptivity of sheep cells for proteins by this method.

When formalinized cells were sensitized to serum proteins and titrated against heterologous serum protein antiserums, some cross reaction was seen. This is perhaps explained by the incomplete separation of serum proteins in fractionation. When cells were sensitized to purified casein (Difco) and titrated against anti-BSA or anti-BGG rabbit serums, no cross reaction occurred.

Summary. 1. A method has been described by which bovine serum albumin and bovine γ-globulin may be coupled to formalinized erythrocytes. 2. These sensitized cells are stable in either the frozen or lyophilized state for at least 5-6 months.

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Studies on Nonspecific Acquired Resistance to Viral Toxicity in Mice.* (23303)

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Results obtained in several laboratories have indicated that prior injection of viable or inactivated influenza virus protects mice to challenge with toxic quantities of virus administered by the same route. The rapidity with which resistance develops, and its production by serologically heterologous virus, preclude the possibility that specific immunity based upon antibody production is involved in such protection. Such protection has been produced in mice both by intracerebral(1,2) and intravenous(3,4) routes and resembles viral interference. On the other hand, susceptibility of experimental animals to bacterial infection or endotoxin has been modified by a number of nonspecific physiological factors(5-8). For example prior injection of bacterial endotoxin protected mice against subsequent bacterial infection or challenge with endotoxin, and was associated with an elevation of the properdin level of the serum (6-7). The present investigation was undertaken to determine what relation, if any, these phenomena might have to one another.

Methods. The PR8 and NWS (neurotropic) strains of influenza A virus, the Lee strain of influenza B virus, and the RO strain of Newcastle disease virus (NDV) were cultivated in the allantoic sac of embryonated eggs. Virus was sedimented in a Spinco model L ultracentrifuge at 78,000 x g, resuspended in 1/10 the original volume of tryptose broth, and stored at –70°C. Technics for demonstrating the toxic effects of intracerebrally injected influenza viruses(9,10) and NDV(11,12) have been described. Protection tests in mice were performed similar to that described by Wagner(1). Weanling Swiss albino mice weighing 9-10 g each were used for intracerebral inoculation. Prechallenge injections were 0.01 ml, while challenge injections made into the opposite hemisphere were 0.03 ml. All intravenous injections were 0.5 ml into the tail vein of mice weighing 18-20 g each. All mice were observed daily for 5 days and necropsied when the experiment was terminated. *Escherichia coli* endotoxin was prepared by the trichloroacetic acid method(13). The LD_{50} of these preparations was approximately 0.18 mg when injected intravenously into 20 g mice.

Results. Table I summarizes the results of 3 experiments showing that prior intracerebral injection of a number of unrelated materials exerted a modifying effect on the neurotoxic effect of influenza A virus. Groups of 20 or 40 mice each were injected intracerebrally with the indicated amounts of ribonucleic acid (RNA), desoxyribonucleic acid (DNA), purified type III pneumococcus polysaccharide, *E. coli* endotoxin, undiluted horse serum, or with saline. RNA and DNA were adjusted to pH 6.8-7.0 with NaOH and were used, as was the pneumococcus polysaccharide, at the limit of solubility. Twenty-four hours later, half of the mice in each

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