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Studies *in Vitro* on Physiology of Cells: Effect of Anisotonic Solutions.*

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The effect of osmotic pressure on erythrocytes has been extensively studied partly due to the intrinsic importance of the red blood cells and partly due to the ease in obtaining a suspension of the cells, in observing their hemolysis and in examining them microscopically by smears or in suspension. As a result of these studies, considerable information has been obtained on the effect of anisotonic solutions on erythrocytes and on the permeability of the cell membrane to various reagents.[1]

The method of unstained cell counts[2] permits analogous studies using cells in suspensions derived from the thymus, testis, and other organs.

Effect of Distilled Water. The methods used for preparing cellular suspensions and for counting the number of viable cells in the suspensions are presented in detail in previous papers.[2] In brief, the thymus or other organ is chopped up with scissors after adding a small amount of Ringer's solution which was buffered with 20% of Sorensen's phosphate solution having a pH of 7.7. This solution is isotonic with the cells and did not affect the size or shape of red blood cells. The cells in the suspension were washed with and then suspended in the phosphate-Ringer solution. To 0.2 ml amounts of suspension were added 0.1 to 0.8 ml of distilled water. The mixtures were shaken 15 minutes in a water bath at 37.5°C. After incubation, a solution of eosin (1:1000 in Tyrode's fluid) was added to make a final volume of 4.0 ml. The presence and character of any precipitate were noted and counts were made of the stained and unstained cells and of the red blood cells. Earlier papers[2] have demonstrated that the unstained cells are, in all probability, viable and that the stained cells are dead. Suspensions of erythrocytes were diluted with Tyrode's solution instead of eosin and the amount of hemolysis was determined after centrifugation. The results are shown in Table I.

The macroscopic observations on the effect of distilled water on cellular suspensions were of considerable interest. Distilled water caused hemolysis in suspensions of erythrocytes, 0.1 ml of water producing partial and 0.4 ml complete hemolysis. No gross changes were observed on the addition of water to suspensions of polymorphonuclear leukocytes and splenic cells. Testicular cells in suspension were clumped by distilled water. The white, opaque clumps were readily broken up by drawing them back and forth in a pipette and the cells were resuspended. On the other hand, the addition of distilled water to suspensions of thymic cells of the rat or rabbit resulted in the precipitation of a large, gelatinous, translucent mass which was not dissolved by the addition of the eosin solution. The greater the number of thymic cells in suspension, the larger was the precipitate. The supernatant was clear when large amounts of water were added (0.4 and 0.8 ml) and was turbid after the addition of smaller volumes of water (0.1 and 0.2 ml).

A few experiments were performed to study the nature of the characteristic precipitate produced in thymic suspensions. The addition of isotonic solutions of sucrose instead of distilled water did not cause precipitation. This finding indicates that the action of distilled water was not due to the dilution of the electrolytes but was rather the effect of the reduced osmotic tension. The addition of distilled water to thymic cells killed by heating at 55°C for one hour also resulted in a pre-

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1 Cowdry, E. V., *General Cytology*, Chicago, University of Chicago Press, 1924.

2 Schrek, R., *Am. J. Cancer*, 1936, **28**, 389; *Arch. Path.*, 1943, **35**, 857; *ibid.*, 1944, **37**, 319; PROC. SOC. EXP. BIOL. AND MED., 1944, **54**, 283.

TABLE I. Toxic, Hemolytic, and Precipitating Action of Distilled Water on Cells in Suspension.

	Ml distilled water added to 0.2 ml of suspension	% distilled water in final susp.	Red blood cells of rabbit	Thymus of rat	Thymus of rabbit	Spleen of rat	Lymphatic leukemia of mouse	Lymphatic leukemia of man	Peritoneal exudate of rat	Testicle of rat
Macroscopic observations	0	0		T	T	T	T	T	T	T
	0.1	33	H 2+	T	T		T	T		T
	0.2	50	H 2+	P 1+	P 1+		T	T	T	T
	0.4	67	H 3+	P 2+	P 1+	T	T	C 1+	T	C 1+
	0.8	80	H 3+	P 2+	P 2+	T	C 1+	C 1+	T	C 1+
No. of unstained cells per millimicroliter	0	0	265	112	110	356	206	181	87	158
	0.1	33	80	110	93		195	207		65
	0.2	50	35	101*	25*		182	225	91	38
	0.4	67	6	49*	3*	195	87	186	31	28
	0.8	80	0	7*	1*	70	28	90	9	13

H = Hemolysis.
T = Homogeneously turbid.
P = Gelatinous precipitate which cannot be resuspended.
C = Clumping of cells which can be broken up.
1+ = Slight degree.
2+ = Moderate degree.
3+ = Marked degree.
* Count is unreliable due to the presence of precipitate.

cipitate which was, however, somewhat opaque instead of translucent and was not as voluminous as in the unheated suspension. In one experiment the mass was washed with phosphate-Ringer solution and then chopped up with scissors and filtered through wire gauze. The resultant suspension was found to contain a small number of cells, some of which were still viable. The precipitate, on histologic section, consisted of a network of thin basophilic fibrils. Slightly enlarged and normal-sized cells, in moderate numbers, were attached to the fibrils. The nuclei of the cells stained deeply and some were indented markedly so that they appeared in the shape of a horseshoe or ring.

The toxicity of distilled water was determined by counting the number of erythrocytes or viable cells (*i.e.*, cells resistant to staining with eosin) after 15 minutes exposure to the water. Table I shows that the cells differed in their susceptibility to the toxic action of distilled water. The addition of 0.2 ml of distilled water to 0.2 ml of suspension resulted in (1) a marked reduction in the number of red blood cells (from 265 to 35), (2) a considerable decrease in the number of viable testicular cells (from 158 to 38), and no appreciable change in the number of polymorphonuclear leukocytes, leukemic cells, and thymic cells of the rat. It was observed, furthermore, that testicular and other cells were lysed by high concentrations of distilled water so that the total numbers of stained and unstained cells in the suspensions were decreased. It may be concluded that distilled water had a more deleterious effect on erythrocytes and testicular cells than on the other cells tested.

Effect of Freezing and Thawing. Experiments on the freezing of cellular suspensions gave results which were to some extent similar to those obtained on the addition of distilled water. A suspension of thymic cells was frozen with solid carbon dioxide for 10 minutes and then thawed in the water bath at 37.5°C. As a result of this procedure, the suspension developed a large, white gelatinous precipitate. A histologic section of the precipitate showed parallel strands of basophilic fibrils to which were attached a moderate

TABLE II. Toxic Effect of Sodium Chloride, Sucrose, Ethyl Alcohol, and Urea in Varying Concentrations.

Solvent	Molarity of reagent	Number of unstained cells per millimicroliter: Thymus of rat: Sodium chloride	Thymus of rat: Sucrose	Thymus of rat: Urea	Thymus of rat: Ethyl alcohol	Testicle of rat: Sodium chloride	Testicle of rat: Sucrose	Testicle of rat: Urea	Testicle of rat: Ethyl alcohol	Peritoneal cells: Urea
Distilled water	0			5*				9		
	0.02	14*				12				
	0.05	53*	65*			20	11			
	0.1	109	100*	7*	12*	159	21	11	25	
	0.2	97	110	11*	12*	153	57	19	27	
	0.5	104	117	10*	18*	85	45	5	25	
	1.	39†	113	7	7*	60	59	3	17	
	2.	2†	11	1	0	51†	44	1	9	
Phosphate-Ringer	0	115	139	230	208	154	83	220	173	108
	0.1			200				177		
	0.2			223	167			72	184	
	0.5			256	188			22	130	92
	1.			201	194			7	221	97
	2.			155	162			4	196	31

* Count is not reliable due to precipitate. † Cells are clumped but can be readily resuspended.

number of lymphocytes. Under identical conditions, suspensions of splenic and testicular cells failed to develop any precipitate. Freezing and thawing, like distilled water, caused a precipitate in thymic but not in the other suspensions.

A few incidental observations were made in the above experiments. The freezing and thawing caused the lysis of the erythrocytes and the death of most of the nucleated cells. However, an occasional lymphocytic and testicular cell remained viable as indicated by its resistance to staining with eosin.

Effect of Sodium Chloride and Sucrose. Various concentrations of sodium chloride and of sucrose in distilled water and in phosphate-Ringer solution were added to cellular suspensions (0.8 ml of reagent to 0.2 ml of suspension). The mixtures were shaken for 15 minutes at 37.5°C and then examined.

It is seen from Table II that sodium chloride and sucrose were relatively non-toxic at certain concentrations (0.1 to 0.5 molar for sodium chloride and 0.2 to 1.0 molar for sucrose) which are isotonic or moderately anisotonic for the cell. The method was not suitable for determining the concentration of isotonic solutions since fluids which were slightly or moderately anisotonic were not lethal to the cell.

Markedly hypotonic solutions of sodium chloride and sucrose decreased the number of viable cells and also caused a gelatinous precipitate in suspensions derived from the thymus. These findings are in accord with those observed on the addition of distilled water.

Markedly hypertonic solutions of the 2 reagents were also found to be toxic to testicular and other cells. The hypertonic solutions of sodium chloride caused clumping of the cells. The clumps could be broken up by drawing them back and forth in a pipette.

Effect of Ethyl Alcohol and Urea. It has been shown that sodium chloride and sucrose in distilled water were relatively non-toxic in certain concentrations. In contrast ethyl alcohol in distilled water was found to be cytocidal at all the concentrations studied (0.1 to 2 molar). It was observed furthermore that alcohol in distilled water caused the same type of gelatinous precipitate in thymic sus-

pensions as was observed on the addition of distilled water alone. On the other hand, ethyl alcohol dissolved in phosphate-Ringer solution had little or no lethal effect on thymic and testicular cells even in high concentrations (2 molar). A 3-molar solution had a marked cytocidal action in fifteen minutes at 37.5°C.

From these experiments, it seems that ethyl alcohol exerts little if any osmotic tension on the cells. This would indicate that the cell wall was readily permeable to the reagent. In spite of its apparent capacity to penetrate into the cell, ethyl alcohol has only a slight cytocidal action.

Similar experiments with urea showed that this reagent also failed to exert an osmotic pressure on the cell. Evidently the cellular wall was also permeable to this compound. Urea in 1 molar solution had no appreciable effect on the number of viable thymic cells or polymorphonuclear leukocytes and did not cause the hemolysis of erythrocytes but did cause a marked decrease in the number of viable testicular cells (from 220 to 7 cells per millimicroliter). Evidently urea had a marked toxic effect on testicular cells but not on the other cells studied.

Summary. By means of the method of unstained cell counts both distilled water and urea were found to be more toxic to testicular cells than to lymphocytes and polymorphonuclear leukocytes. Ethyl alcohol was not cytocidal even in relatively high concentrations. The cell membrane of viable thymic, testicular and other cells appeared readily permeable to urea and ethyl alcohol but not to sodium chloride or sucrose. The addition of distilled water or freezing and thawing produced a characteristic gelatinous precipitate only in suspensions of thymic cells but not in suspensions of splenic or testicular cells.

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Effect of Penicillin on Certain Viruses.

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In view of the wide antibiotic activity of penicillin, it is of interest to learn whether it has any effect on the growth of viruses. The present paper deals with experiments designed to gain information on this subject.

Certain representative viruses were used as follows: Vaccinia BH,[1] a strain of vaccine virus derived originally from the New York Board of Health Strain and maintained for several years by rabbit testicular passage; Vaccinia CV II,[1] derived from BH by serial passage in Rivers-Li culture medium now of apparently stable virulence; St. Louis Encephalitis, Hubbard strain, obtained from Dr. Margaret E. Smith, and maintained by serial intracerebral mouse passage; Psittacosis 6 BC, obtained through the courtesy of Dr. J. E. Smadel, maintained by serial yolk sac passages; Meningo-pneumonitis virus, M.P., obtained from Dr. T. Francis, Jr., and maintained by mouse lung and chick embryo yolk sac passage; Equine Encephalomyelitis virus, the Eastern strain, secured through the courtesy of Dr. J. E. Beard, was used in its third chick embryo passage.

Penicillin used was prepared by the Reichel and Abbott Laboratories and was part of a supply allocated to the University Hospitals of Cleveland by the Committee on Therapeutics and Other Agents of the National Research Council. Assays were made by the Oxford cup method using spores of *Bacillus subtilis* for inoculating the solid medium as suggested by Foster and Woodruff.[2] It was found that

[1] Parker, R. F., Bronson, L. H., and Green, R. H., *J. Exp. Med.*, 1941, **74**, 263.

[2] Foster, J. W., and Woodruff, H. B., *J. Bact.*, 1944, **47**, 43.