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Modifications of the Rous-Turner Solution for Preservation of Bird Erythrocytes.*

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A concentration of 0.7% NaCl has been found by one of us (M.R.I) to be satisfactory for keeping the erythrocytes of various species of birds for relatively short periods of time, one to 2 weeks. In the hope of lengthening the time of survival of these cells, and at the same time retaining their agglutinability, modifications of the Rous-Turner formula[1] for mammalian cells were attempted. Cells from a species of dove *(Streptopelia risoria)*, of pigeon *(Columba livia)*, and of the domestic chicken *(Gallus gallus)* were used in about a 5% suspension with varying strengths of solutions.

A dilution of the Rous-Turner formula (9.2 gm. NaCl, 0.05 gm. $NaHCO_3$, 0.1 gm. KCl, 0.1 gm. $CaCl_2$) approximately isotonic with 0.7% NaCl was made by adding 32 cc. of distilled water to 100 cc. of the solution. For the trials, 100 cc. of the solution was diluted by the addition of from 28 to 34 cc. of distilled water. Equal parts of dextrose (Bacto) solution were added, in a concentration of 5.4% dextrose as recommended by Rous,[1] and, also, 4.2% dextrose, which was an amount arbitrarily taken as being proportional to the original solution of saline suitable for the preservation of bird cells. Only sterile solutions were used. These were tested in duplicate with each of the 3 types of cells.

The dextro-saline solution readily supported bacterial growth, even at refrigerator temperatures, making it necessary to collect the blood under as sterile conditions as possible. A satisfactory procedure consisted in first sponging the skin area over the wing vein with tincture of iodine, then with alcohol. The blood, in quantity to approximate a 5% suspension, was added directly to the tubes (16 or 18 mm. dia.) containing the different solutions. The mere dilution of the blood prevented an appreciable amount of clotting. For bloods not obtained with the above precautions, the addition of merthiolate even in 1:1000 was found to be insufficiently bacteriostatic for preservation purposes.

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1 Rous, P., and Turner, J. R., *J. Exp. Med.*, 1916, **23**, 219.

TABLE I.
Preservation of Erythrocytes from Different Species of Birds in Various solutions.

	Weeks	1	2	3	4	5	6	7	12
.7% NaCl	Chicken	++	+++	+++	++++	C			
	Pigeon	—	+++	+++	+++	C			
	Dove	+	++	++	+++	+++	++++	C	
Locke's Solution	Chicken	—	+	++	+++	C			
	Pigeon	—	+++	+++	+++	C			
	Dove	+	+++	+++	++++	++++	++++	C	
100 cc. Locke's Sol. + equal parts 5.4% dextrose	Chicken	—	—	—	—	—	—	—	+
	Pigeon	—	+++	+++	+++	C			
	Dove	—	±	±	+	++	++	+++	C
.7% NaCl + equal parts 5.4% dextrose	Chicken	—	—	—	—	—	—	—	+
	Pigeon	—	+	++	++	+++	C		
	Dove	—	±	±	+	+	++	+++	++++
100 cc. Locke's Sol. + 30 cc. dist. H_2O + equal parts 4.2% dextrose	Chicken	—	—	—	—	—	—	—	±
	Pigeon	—	±	+	++	+++	+++	++++	
	Dove	—	—	—	—	±	+	++	
100 cc. Locke's Sol. + 32 cc. dist. H_2O + equal parts 5.4% dextrose	Chicken	—	—	—	—	—	—	—	±
	Pigeon	—	±	+	+	+	+	++	
	Dove	—	—	—	—	+	+	++	
100 cc. Locke's Sol. + 32 cc. dist. H_2O + equal parts 4.2% dextrose	Chicken	—	—	—	—	—	—	—	±
	Pigeon	—	±	±	+	+	+	++	
	Dove	—	—	—	—	±	+	++	
100 cc. Locke's Sol. + 34 cc. dist. H_2O + equal parts 5.4% dextrose	Chicken	—	—	—	—	—	—	—	—
	Pigeon	—	—	—	—	±	±	+	
	Dove	—	—	±	±	+	++	+++	

Symbols: —, no lysis; ±, very faint trace; ±, faint trace; +, trace; ++, +++, ++++, well defined lysis in increasing amounts, respectively; C, complete lysis.

The tubes were stored at 3-5°C., and were examined for lysis at intervals of 7 to 9 days, at which time the tubes were shaken to assure thorough mixing. Lysis was measured roughly by the intensity and amount of coloration of the supernatant fluid.

The findings of 2 independent trials are summarized in Table I. These tests, although not giving identical results, agreed so closely that they are grouped together. Only those solutions with which favorable *results* were obtained in preserving the cells are given in the table. The other solutions not included gave in general less successful preservation of the pigeon and dove cells, and to some extent of the chicken cells. The addition to these various solutions of ¼ or ½% gelatine was of no aid in preventing lysis.

It is readily apparent in the table that chicken cells are much more easily preserved in dextrose solutions than those of either pigeon or dove, both as to length of time and in the range of dilutions possible. Furthermore, there is some indication that, in many of the dilutions given, dove cells were preserved longer than pigeon cells. The exception, in the solution containing 34 cc. of distilled water plus 5.4% dextrose, was observed in each of the separate trials. All these cells, however, were fairly well preserved in any one of the following combinations: (a) 100 cc. of Locke's solution plus 32 or 34 cc. of distilled water, (b) to which was added equal parts of either 4.2% or 5.4% of dextrose solution.

The agglutinability of the suspended cells with specific antisera was tried at the 6th week in the first test, and at the 8th week in the second. In all cases, the stored cells agglutinated at the same end dilution of the antisera as did fresh cells of each species. Whether or not each of the many antigens present in these cells[2, 3] will be fully expressed in like manner after standing is yet to be determined.

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Hyperglycemia Due to Impaired Hepatic Glycogenesis.

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It has been recognized that there is a difference in many respects between the severe type of diabetes mellitus occurring in the child

2 Irwin, M. R., and Cole, L. J., *J. Exp. Zool.*, 1936, **73**, 85; 1936, **73**, 309.

3 Todd, Chas., *Proc. Royal Soc. (B)*, 1930, **106**, 20; 1931, **107**, 197.