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Research Article

EFFECT OF INOSINE ON THE POST-TRANSFUSION SURVIVAL OF STORED RABBIT ERYTHROCYTES *

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In 1955 Gabrio, Donohue, Huennekins and Finch (1) reported that inosine prolonged the period that human blood could be stored at 4° C. in acid citrate dextrose (ACD); their experimental evidence indicated greater than 70 per cent viability of the erythrocytes after six weeks of storage. Because of these encouraging findings, a cooperative study was undertaken in which the post-transfusion survival of erythrocytes from blood stored in ACD and in ACD plus inosine (ACDI) was compared (2). Results of this study were not as encouraging as those previously found, and it was reported that only four of the 23 units of blood stored for six weeks in ACDI had better than 70 per cent post-transfusion survival of the erythrocytes. Because of these rather inconsistent results the present study was instituted to learn more about factors which might influence the effect of inosine on the stored erythrocyte. To facilitate a large number and variety of tests, rabbit blood was used since previous work indicated that the results probably would be applicable to human blood.

METHODS

Blood was drawn from one or two female New Zealand rabbits (depending on the quantity of blood to be stored) by intracardiac puncture with heparinized syringes, added to ACD, transferred to siliconized flasks in 5 or 10 ml. volumes, and stored for six weeks at 4° C. Sterile precautions were observed throughout the storage period. Inosine was autoclaved in normal saline unless otherwise stated. In each experiment, ACD blood was stored without inosine as a control and with inosine under various experimental circumstances. At the end of the storage period, post-transfusion survival of the erythrocytes was determined by the double isotope (Cr^{51} and P^{32}) method as described by Gibson and Scheitlin (3). A separate female New Zealand rabbit was used for each post-transfusion survival study.

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RESULTS

Tables I through IV give the 24 hour post-transfusion survival results for erythrocytes of the blood stored six weeks at 4° C. in ACD alone (Table I), and in ACD supplemented with inosine (Tables II through IV).

Fifteen control blood samples stored in ACD alone gave erythrocyte post-transfusion survival values ranging from 4 to 33 per cent with a mean of 18 per cent and a standard deviation of 7.5. Post-transfusion survival of erythrocytes from 49 samples of blood stored in ACDI varied from 28 to 100 per cent with a mean of 61 per cent and a standard deviation of 22. Thus, on the average,

TABLE I

Twenty-four hour post-transfusion survival of erythrocytes stored six weeks at 4° C. in acid citrate dextrose (ACD)

Sample	% 24 Hr. post-transfusion survival	Sample	% 24 Hr. post-transfusion survival
1	9	9	23
2	20	10	25
3	12	11	33
4	12	12	17
5	11	13	23
6	17	14	25
7	20	15	27
8	4	Mean	18
		Standard deviation	±7.5

TABLE II

Effect of inosine on blood stored six weeks at 4° C. in ACD; variation of amount and of time of addition of inosine*

Inosine mg./ml. ACD-blood	Time of addition	% 24 Hr. post-transfusion survival
4.0	0 day	65
4.0	0 day	73
4.0	0 day	93
4.0	21 days	55
4.0	21 days	87
2.0	0 day	72
0.4	Semiweekly	100
0.2	Semiweekly	59

* Schwarz Laboratories, Inc., Lot No. 5504.

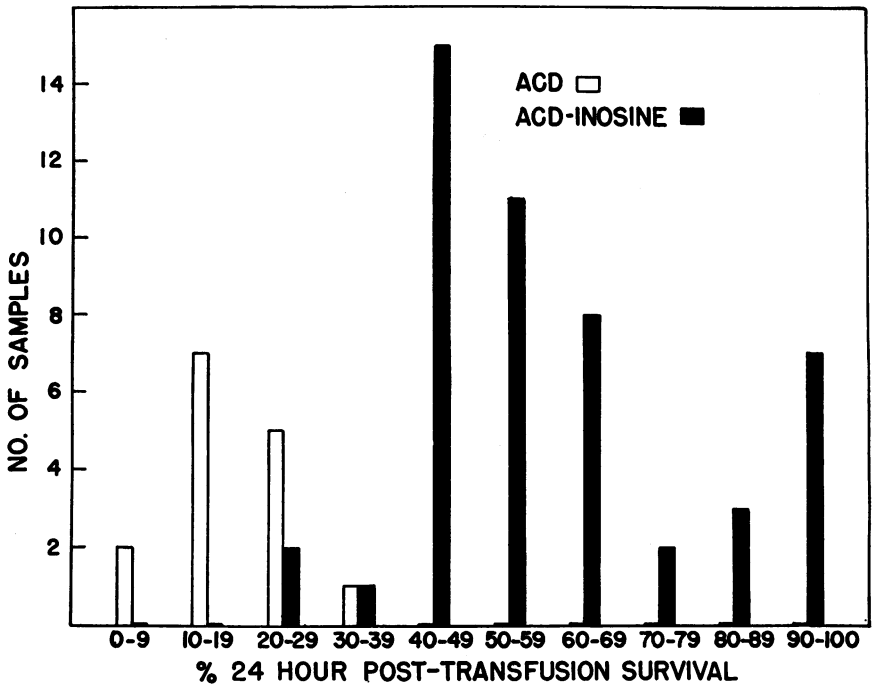

FIG. 1. INCIDENCE GRAPH OF 24 HOUR POST-TRANSFUSION SURVIVAL OF ERYTHROCYTES STORED FOR SIX WEEKS AT 4° C. IN ACID CITRATE DEXTROSE (ACD) AND IN ACD-INOSINE

Mean 24 hour post-transfusion survival of erythrocytes from 15 samples of blood stored in ACD was 18 per cent ± 7.5, and from 49 samples of blood stored in ACD-inosine was 61 per cent ± 22.

3.4 times as many erythrocytes were viable after six weeks of storage when ACD was supplemented with inosine. The results are shown graphically in Figure 1.

The post-transfusion survival of erythrocytes from several samples of stored blood was followed past 24 hours and the curves, presented in Figure 2, show, in agreement with other investigators, that nonviable cells are rapidly removed from the circulation, and that those remaining after 24 hours disappear at approximately a normal rate.

TABLE III

Effect of inosine on blood stored six weeks at 4° C. in ACD; variation in autoclaving and formula of ACD*

Inosine mg./ml. ACD-blood	ACD formula	Inosine solution autoclaved	% 24 Hr. post-transfusion survival
4.0	B	Normal saline	52
4.0	B	Normal saline	69
4.0	B	ACD	52
4.0	B	ACD	66
4.0	A	Normal saline	35
4.0	A	Normal saline	81
4.0	A	ACD	53
4.0	A	ACD	69

* Schwarz Laboratories, Inc., Lot No. 5504.

The following variables were investigated: *1*) addition of inosine at various times during the storage period, *2*) periodic shaking of the blood, *3*) autoclaving inosine in ACD or in normal saline, and *4*) addition of different lots of inosine manufactured by several suppliers. There were no clear-cut differences which would allow the conclusion that any of these factors significantly influenced erythrocyte viability during storage.

Samples of inosine were examined on columns of Dowex-1 formate after autoclaving in ACD or in normal saline. In both cases the compound was eluted quantitatively as a sharp band in the position expected, analyzed correctly by ultraviolet absorption and the orcinol method, and therefore showed no evidence that any breakdown of the nucleoside was produced.

TABLE IV

Effect of inosine (4 mg. per ml. ACD-blood) on blood stored six weeks at 4° C. in ACD; variation of inosine preparation

Inosine preparation	% 24 Hr. post-transfusion survival	Inosine preparation	% 24 Hr. post-transfusion survival
S-5705*	44	S-5504	66
S-5705	49	S-5504	60
S-5601	40	S-5504	65
S-5601	51	S-5706	49
S-5602	40	S-5706	65
S-5602	57	S-5706	48
S-5605	40	S-5706	51
S-5605	46	S-Crude†	41
S-5606	43	S-Crude	84
S-5606	58	S-Crude	95
S-5702	28	S-Crude	97
S-5702	56	P-5706‡	100
S-5703	40	P-5706	92
S-5703	49	P-2303	100
S-5704	40	P-2303	29
S-5504	48	C-100898§	40
		C-100898	52

* Schwarz Laboratories, Inc.
† Schwarz Laboratories, Inc., unpurified inosine preparation.
‡ Pabst Laboratories.
§ California Corporation.

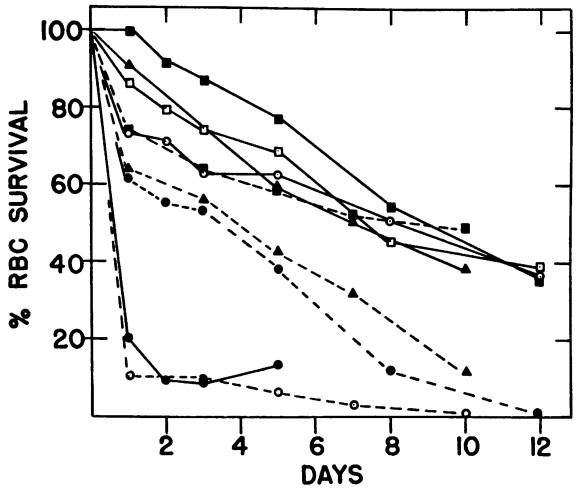

FIG. 2. POST-TRANSFUSION SURVIVAL OF ERYTHROCYTES STORED SIX WEEKS IN ACD AND IN ACD-INOSINE (Cr^{51} AND P^{32} METHOD)

Graphic representation of per cent survival of calculated erythrocytes transfused, O - - - - O and ●———● —control blood samples stored in ACD. Others, blood samples stored in ACD-inosine.

DISCUSSION

The results are distinguished by a marked variability in the survival of the erythrocytes stored in ACD either with or without inosine. It is not possible to ascertain from these experiments how much of the variation was due to the blood itself and how much to some influence of the experimental manipulations. It will be difficult to assess any of these inconstant effects of inosine or other changes in incubation solution or technic without more information about erythrocyte viability after extended storage periods in ACD alone.

It is obvious, nevertheless, that inosine has a favorable influence on the survival of the rabbit erythrocyte when stored in ACD, and it is felt that further investigation of the effect of various nucleosides on stored blood is warranted.

SUMMARY

Twenty-four hour post-transfusion survival of erythrocytes was determined on 64 samples of blood stored for six weeks at 4° C. in acid citrate dextrose (ACD) or in ACD plus inosine (ACDI). Erythrocyte post-transfusion survival values of 4 to 33 per cent and 28 to 100 per cent for blood stored in ACD and in ACDI, respectively, were obtained.

Inosine has a beneficial effect on the viability of stored blood although there is considerable variability in this effect.

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